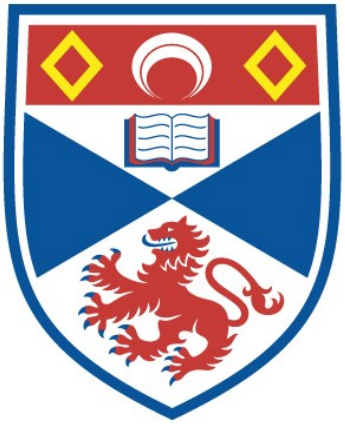
BEHAVIOURAL EXAMINATION OF THE ROLE OF THE THALAMIC RETICULAR NUCLEUS IN ATTENTION

Rudi Stanislaus-Carter

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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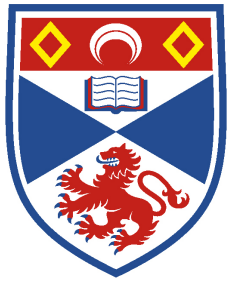

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This thesis is submitted in partial fulfilment for the degree of PhD at the University of St Andrews

September 2016

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Publications and Presentations

The work detailed in this thesis resulted in the following poster presentation:

Poster presentation, Society for Neuroscience, San Diego CA “Bilateral 6-hydroxydopamine lesions of the thalamic reticular nucleus impair the re-orientation of attention”.

Abstract

The ability to selectively attend to aspects of the environment which signal opportunity or danger, while marginalising irrelevant stimuli is critical to an animal's survival. With finite cognitive resources, the brain must dedicate resources to only those stimuli that are biologically significant. Incoming thalamic information must therefore be filtered. The thalamic reticular nucleus has long been considered critically involved in modulating thalamic sensory processing. Sharing connections with both the thalamus and cortex, it is ideally located to modulate the transfer of pertinent incoming sensory information.

This thesis sought to determine the functional role of the thalamic reticular nucleus in attentional processes by combining lesion techniques and well established behavioural paradigms.

Chapter 3 examined the role of visual thalamic reticular nucleus lesions on performance in a two-alternative forced choice reaction time task when auditory distractors were presented. No effect of the lesion was found. Chapter 4 examined excitotoxic lesions of thalamic retlcular nucleus on performance in the 7-stage attentional set shifting task. No effect of lesion on performance was found. Chapter 5 examined mediodorsal thalamus and rostral thalamic reticular nucleus lesions on performance in the attentional set shifting task. Despite strong connectivity with prefrontal regions known to be involved in this task, there was no effect of either lesion. Finally, chapter 6 examined the effects of reducing dopamine input into the thalamic reticular nucleus on a two alternative forced choice reaction time task. Following bilateral lesions the animals were impaired in the re-orientation of attention – suggesting a critical role for both the thalamic reticular nucleus and

dopamine in attentional processes. Taken together, these results suggest that while the thalamic reticular nucleus is involved in attention, it is not involved in every aspect.

Introduction

1.1 Attention Introduction

Attention is a ubiquitous concept, pervading many areas of research. It can be defined as the activation of neural networks and mechanisms that aid in the selection of relevant sensory information often at the expense of other present stimuli. This information is then used to control behaviour in the current task as based upon the current drives or goals of the said animal (Wenk, 1997). Not all sensory information that an animal encounters is used to inform and control any subsequent behaviour. The animal must therefore be able to 'select' or differentiate between aspects of their environment that confer beneficial information and those that are superfluous/redundant. It is evident that information selected is that which the animal believes to be the most efficacious predictor of proceeding events. This is predicated on the ability of the animal to decipher relevant from irrelevant information, and is defined by some, including Quinlan (2008), as "a form of act selection" (p. 218). This definition is appropriate because of all the information which initially receives sensory processing only a subset is selected and therefore acted upon. It could be argued that this 'selection' underpins what is considered attention. In selecting which information shall be attended to, the animal has thus begun to shape their behaviour: only that receiving consideration can be used when determining how to act (Mackintosh, 1975).

The allocation, or focusing, of attention is often dichotomised into two separate processes – bottom up and top down. However one must be aware that

while they are viewed as two different processes, they recruit the same brain areas; the only difference being the stage at which the area becomes involved in processing and/or behaviour. Firstly, there is top-down, endogenous control whereby attention is focused voluntarily based upon prior knowledge about of the nature of the task. Secondly, there is the somewhat cruder bottom-up, exogenous processing whereby attention is directed by properties that are inherently salient within the stimulus itself – for example the sounding of a fire alarm (Buschman & Miller, 2007). Bottom-up perspectives of attention attempt to explain the ability to detect targets as being a sole consequence of its inherent importance or salience, which subsequently results in it recruiting 'higher cortical' areas for additional processing.

Top-down control of attention has been shown to be most beneficial in terms of stimuli processing time (Kastner & Ungerleider, 2000). Allocating attention and determining/preparing the behavioural response prior to stimulus onset allows the animal to exhibit the desired behaviour quicker which, from certain perspectives, could be beneficial to survival (Bradley, 2009). One can break down the voluntary orientation of attention into several mechanisms: disengaging attention from the stimulus currently focused upon, reorienting attention to the new locus of interest, and finally selectively processing inputs from stimuli and maintaining the attentive state. The first two stages could be viewed as top-down control, with the final stage of selective processing of sensory input being the consequence of this top down control (Hopfinger, Buonocore, & Mangun, 2000).

It was initially believed that the thalamus was merely a relay for sensory information, and played little or no role in attention. However, the strategic position of the thalamus and its connectivity with cortex suggest otherwise. All incoming

sensory information, aside from olfaction, goes through thalamus, with subsets of the incoming information proceeding to cortex for additional processing (for details of olfactory processing see Mori, Nagao, Yoshihara, 1999; Shepherd, 2005). Any area(s) implicated in complex functions that are reliant on the manipulation of sensory stimuli need to have a detailed and accurate representation of the external world. This topographical organisation would allow for the manipulation of certain aspects of information processing without – if necessary – interfering with other points of communication/sensory transmission (Sherman and Guillery, 1996; McAlonan & Brown, 2002).

It was first proposed that the thalamic reticular nucleus was merely a continuation of the reticular formation of the brain stem, utilising diffuse widespread projections and therefore exerting global rather than specific actions (Brodal, 1981). However, both dorsal and ventral thalamic nuclei have topographic maps for all sensory domains except olfaction. These sensitive topographic maps permit "building" of full body representations of incoming sensory stimuli which can then be manipulated. Manipulations of this incoming sensory flow can be used to direct behaviour, and optimise interaction with the environment.

1.2 The Thalamus

1.2.1 Dorsal Thalamus

In recent years it has become apparent that the thalamus has much more functional significance than its derivation suggests. From the Greek for chamber (θάλαμος), it was previously viewed as merely a vessel allowing the passage of sensory information en route to cortex. The dorsal thalamus has a detailed

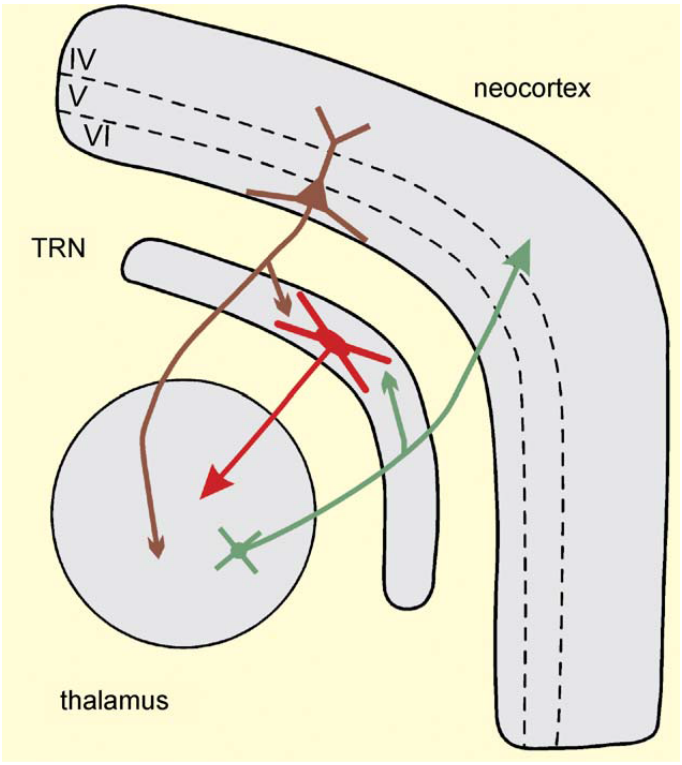
topographic map representing all sensory surfaces except olfaction and has been referred to as the gateway to cortex for sensory input (Crick, 1984).

A plethora of studies, ranging from electrophysiological to behavioural have shown that the nuclei of the thalamus can be divided into first and higher order (for analysis of the literature see Mitchell, Sherman, Sommer, Mair, vertes, & Chudasama, 2014; Mitchell, 2015). While first order thalamic nuclei are more involved in initial incoming sensory information originating directly from the periphery (with the exception of olfaction), higher order thalamic nuclei receive little direct sensory information. Rather, higher order thalamic nuclei are involved in the further processing of this information and transmission of information from one higher cortical area to another. For example, both the lateral geniculate nucleus and the pulvinar are thalamic nuclei that are involved in visual processing (Chen, Kato, Zhu, Ogawa,Tank, & Ugurbil., 1998; O'Connor, Fukui, Pinsk, & Kastner, 2002; Kaas & Lyon, 2007). If the thalamus was merely just a relay of sensory information to the cortex, then the actions of the first order lateral geniculate nucleus would render pulvinar redundant. We know this is not the case, with the pulvinar implicated in the processing and transmission of visual information from multiple thalamic and cortical streams (Grieve, Acuña, & Cudeiro, 2000; Casanova, Merabet, Desautels, & Minville; 2001; Saalmann, Pinsk,Wang, Li, & Kastner, 2012). Distinctions in firing patterns between first and higher order thalamic relays have also been documented, with greater bursting recorded in higher order relays (Guillery, Feig, & Van Lieshout, 2001; Ramcharan, Gnadt, & Sherman, 2005).

In recent years it has been shown that lesions of higher order thalamic nuclei result in memory and attention impairments, strengthening the view that the

thalamus is more than a passive relay (Saalman & Kastner, 2011; Jankowski et al, 2013). Firing activity in higher order relays has been correlated with behavioural states, with greater burst firing at times when the animal is in a state of alertness or attentiveness (Guillery, Feig, & Van Lieshout, 2001; Ramcharan, Gnadt, & Sherman, 2005).

1.2.2 Ventral Thalamus



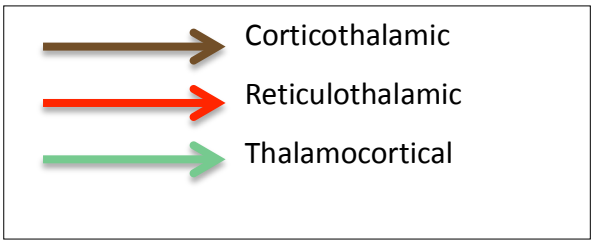


Figure 1.1 . A figure depicting the three modes through which the thalamic reticular nucleus communicates with both dorsal thalamus and cortex. Adapted from Pinault (2004).

A major subdivision of the ventral thalamus is the thalamic reticular nucleus (See figure 1). Within the remit of this thesis, when the term “thalamus” is used either alone or accompanied by more specific location information within the nucleus, it shall refer to sectors of the dorsal thalamus, with discussions of the ventral thalamus limited to the thalamic reticular nucleus.

Unlike the dorsal thalamus, the ventral thalamus does not have direct connections with cortex, instead influencing the transmission of sensory information to cortex by manipulating dorsal thalamic activity (Zikopoulos,& Barbas, 2007; Ferrarelli, & Tononi, 2011). The thalamic reticular nucleus is the major source of inhibition of thalamic activity, with topographic projections to all sensory thalamic nuclei. It is these connections that have led many to hypothesise that the thalamic reticular nucleus is involved in attention processes by filtering incoming sensory information. By inhibiting thalamic activity in one “area” and therefore halting its transmission to cortex, more salient/relevant information receives priority processing.

In addition to connectivity with specific sensory sectors, the thalamic reticular nucleus is involved in synchronising activity of the entire dorsal thalamus (Landisman, Long, Beierlein, Deans, Paul, & Connors, 2002; Long, Landisman, & Connors, 2004). The thalamic reticular nucleus is additionally implicated in the generation of spindles and regulating oscillatory activity, with some labelling the nucleus as a pacemaker (Fuentealba and Steriade, 2005). Spindles are characteristic of early sleep, with abnormal oscillatory activity being a hallmark of spike wave seizures (Steriade, 2005).

It was once believed that the thalamic reticular nucleus was, like the dorsal thalamus, just a passive relay for information. Jones (1975) suggested it was "likely that the reticular complex samples thalamo-cortical and cortico-thalamic activity in a somewhat unspecific manner" (page 285). Early researchers focused on reticular dendrites which had been shown by Scheibel & Scheibel (1966) to stretch along the nucleus plane and across distinct sectors. Placing emphasis on this criterion could easily lead one to believe it unlikely that thalamic reticular nucleus cells could selectively receive topographically organised inputs from a limited number of thalamic nuclei.

Although at the time Jones (1975) believed that the thalamic reticular nucleus was a diffuse nucleus, with hindsight his data showed that the thalamic reticular nucleus has topographically organised inputs and outputs. Injections of radiolabelled amino acids into both the cerebral cortex and dorsal thalamus of rats, cats and monkeys revealed that corticothalamic and thalamocortical fibre bundles traversed the thalamic reticular nucleus. Each bundle, corresponding to a distinct region of both the cortex and thalamus, traversed the thalamic reticular nucleus in the same fashion, thus indicating a topographical organisation consistent with both regions. Furthermore, injections of horseradish peroxidise into thalamic nuclei induced retrograde labelling only in those areas of the thalamic reticular nucleus that received afferents from the same thalamic nucleus (Jones, 1975).

As previously mentioned, any region involved in the manipulation of incoming sensory information requires a topographic map (Kaas, 1997). With the exception of olfaction, the thalamic reticular nucleus has a topographic organisation of all sensory domains (Guillery, Feig, & Lozsadi, 1998).The location and connectivity

of the thalamic reticular nucleus means it is strategically placed to intercept, monitor and control the transmission of sensory information to the cortex (Ferrarelli & Tononi, 2011). This manipulation of sensory information is believed to form the basis of attention. By manipulating which information is transmitted to cortex, there is a selective processing of salient information at the expense of irrelevant information. Crick (1984) was one of the first to propose that the thalamic reticular nucleus was involved in attention in his development of the searchlight hypothesis. He claimed that if all sensory information en route to cortex had to pass through the thalamus, or the gateway, then it was the thalamic reticular nucleus that was the guardian of said gateway controlling the flow.

1.2.3 Anatomy of the thalamic reticular nucleus

1.2.3.1 Caudal thalamic reticular nucleus

The caudal thalamic reticular nucleus is the sensory sector of the area, sharing connections with sensory thalamic nuclei and their corresponding sensory cortical areas (Jones, 1975). All axons from cortex that send projections to the thalamus also send collaterals to the corresponding sector thalamic reticular nucleus (Cornwall & Phillipson, 1988 ; Bourassa et al , 1995). The reticular structure of the thalamic reticular nucleus is consistent with that of thalamus and cortex, with individual sensory sectors for visual, auditory, somatosensory, and gustatory processing.

1.2.3.2 Visual thalamic reticular nucleus

Axons from cortical layer VI projecting to the dorsal lateral geniculate nucleus additionally branch off and traverse the caudal thalamic reticular nucleus of the rat. More specifically, upper layer VI cells project exclusively to caudal thalamic reticular

nucleus and the dorsal lateral geniculate nucleus, while those in the lower aspect of layer VI project to the lateral posterior nuclei of thalamus as well as dorsal lateral geniculate nucleus and caudal thalamic reticular nucleus (Bourassa & Deschênes, 1995). In cats, the visual sector of the thalamic reticular nucleus is detatched from the rest of the nucleus and referred to as the perigeniculate nucleus. Fitzgibbon (2002) demonstrated a topographic organisation of the perigeniculate nucleus, with regions dominated by contralateral eye labelling – consistent with that seen in cat visual cortex (Anderson, Olavarria, and Van Sluyters, 1988).

Labelling of discrete visual thalamic reticular nucleus areas has been detailed in the rat, with different higher order cortical projections occupying areas with little or no overlap (Coleman & Mitrafonis, 1996). Topographic organisation within each modality was additionally shown in the rabbit. Injections into visual areas 1 and 2 resulted in labelling restricted only to the dorsocaudal sector or the thalamic reticular nucleus. Further comparison of two distinct injection sites in visual area 1 (medial and lateral) revealed a medial to lateral shift in labelling both in the visual thalamic reticular nucleus and dorsal lateral geniculate nucleus. In the same fashion, dorsal to ventral shifts in dorsal lateral geniculate nucleus labelling corresponded with dorsal to lateral shifts in thalamic reticular nucleus labelling (Crabtree & Killackey, 1989). The consistent shifts in labelling reflect a topographic map that is a continuation of thalamic and cortical mapping, rather than a diffuse organization that was initially expected in the thalamic reticular nucleus.

The visual sector of the thalamic reticular nucleus can be categorized based upon its connectivity to thalamic areas that are functionally specified for first or higher order processing. Differences in burst firing between visual thalamic reticular

nucleus neurons projecting to first (dorsal lateral geniculate nucleus) and higher order (lateral posterior nuclei) have been documented. Cells projecting to first order thalamic nuclei show a greater propensity for burst spiking, with bursts of larger numbers and shorter interspike intervals in comparison to cells projecting to lateral posterior nuclei (Kimura, Yokoi, Imbe, Donish, & Kaneoke, 2012). This is in contrast with differences in burst spiking between dorsal lateral geniculate nucleus and lateral posterior nuclei, with greater spiking seen in higher order nuclei (Ramcharan, Gnadt, & Sherman, 2005). It is suggested that these differences could occur due to reciprocal connectivity between visual thalamic reticular nucleus and both thalamic nuclei.

1.2.3.3 Auditory thalamic reticular nucleus

The auditory sector thalamic reticular nucleus is ventral to the visual sector. It has been shown that electrical stimulation of the auditory cortex activates the auditory sector of the thalamic reticular nucleus in rats (Yu, Meng, Xu, & He, 2011) , with tracers injected into the auditory sector of the thalamic reticular nucleus labelling neurons in the medial geniculate nucleus (Montero, 1983).

Studied extensively in *Galago*, it was shown that while secondary auditory nuclei (magnocellular division of the medial geniculate nucleus and posterior nucleus) project to the lateral, medial and ventral borders of the auditory thalamic reticular nucleus, the primary auditory nucleus (ventral division of the medial geniculate nucleus) only projects to its central region (Conley, Kupersmith, & Diamond, 1991). Single injections of retrograde tracer into the medial geniculate nucleus of cats resulted in labelling only within the caudoventral sector of the

thalamic reticular nucleus. More specifically, injections restricted to the ventral portion of the medial geniculate nucleus labelled the outer two thirds, and dorsal third of the auditory thalamic reticular nucleus. Injections originating from the ventral two thirds of the ventral sector labelled cells within the ventral two thirds of the auditory thalamic reticular nucleus. Similarly, injections into the magnocellular portion of the medial geniculate nucleus labelled cells restricted to the ventral two thirds and outer two thirds of the auditory thalamic reticular nucleus (Crabtree, 1998).

Injections of tracers into primary auditory cortex and posterodorsal auditory area of rats resulted in strong labelling in caudal thalamic reticular nucleus. Slab like projections were found, with anterograde labelling from injections in primary auditory cortex identifying labelled regions of lateral and middle tiers of ventral thalamic reticular nucleus, while posterodorsal injections labelled more dorsal regions (Kimura, Donish, Okamoto, & Tama, 2005).

In a manner similar to that seen in the visual sector, the auditory thalamic reticular nucleus is divided into distinct tiers, each receiving differential inputs relative to first and higher order processing. Magnocellular medial geniculate nucleus and posterior nucleus connectivity reflect higher order auditory processing, while ventral medial geniculate nucleus connectivity reflects first order auditory processing.

1.2.3.4 Somatosensory thalamic reticular nucleus

Sugitani (1979) was the first to experimentally examine the somatosensory sector of the thalamic reticular nucleus of the rat. By electrically stimulating the

somatosensory cortex, neurons in the thalamic reticular nucleus were identified that only responded to somatosensory input. These neurons surrounded the anterior and anterolateral parts of the ventrobasal complex, known to be the first order relay for somatosensory information. Individual neurons were shown to respond to single stimulation shocks to medial lemniscus, and somatosensory cortex. They were additionally shown to be responsive to natural stimuli that were applied to the skin, joints, hairs and vibrissae (Sugitani, 1979). Additionally, neurons from ventrobasal thalamus projecting to the internal capsule collateralise with the thalamic reticular nucleus (Harris, 1987). Similar findings were documented in the cat, with peripheral stimulation eliciting activity in neurons surrounding the ventrobasal complex (Andersen, Eccles, & Sears, 1964).

More detailed analysis by Crabtree (1992) showed that the somatosensory thalamic reticular nucleus has a distinct “slab” like topographic organisation of projections from somatosensory cortex. Injections from a single site of somatosensory area 1 labelled a specific centroventral region of the nucleus adjacent to the ventrobasal complex. The distribution of terminals from somatosensory cortical area 1 was shown to be similar to those seen in ventrobasal areas. Additional slab like projections in the centroventral sector of the thalamic reticular nucleus were found to originate from somatosensory area 2. These data, obtained from the cat, shows a contralateral mapping of the body surface, hindlimb, forelimb, head and tongue arising from projections from cortical areas (Crabtree, 1992).

Further studies in the rat revealed two “compartments” with different reciprocal connections: a thick lateral sector that is connected to the ventrobasal

complex for first order processing, and a thinner sector related to posterior thalamic nuclei for higher order processing (Pinault, Bourassa, & Deschênes, 1995). Similar results were obtained in the cat, although the projections to the posterior nuclei were not as topographically organised as those seen in the vebtrobasal complex (Crabtree, 1996).

More recently, a third tier of the somatosensory thalamic reticular nucleus has been identified. Recordings from thalamocortical slices revealed that each tier receives distinct input from thalamic somatosensory areas – the posterior medial, ventroposterior medial, and ventroposterior lateral thalamic nuclei. Approximately one quarter of neurons recorded from received input from more than one thalamic source, suggesting a more complex inhibition of somatosensory information than previously thought.

1.2.3.5 Gustatory thalamic reticular nucleus

The gustatory sector of the thalamic reticular nucleus was first identified by Hayama, Hashimoto and Ogawa (1994). Injections of neural tracers horseradish peroxidase, conjugated to wheat germ agglutinin, and biocytin into the parvicelluar part of the thalamic posteromedial ventral nucleus, known as the gustatory thalamic relay, resulted in strong labelling in the ventromedial thalamic reticular nucleus. This sector of the thalamic reticular nucleus surrounded most aspects of the parvicelluar part of the thalamic posteromedial ventral nucleus. Additionally, injections of the same tracers into the cortical gustatory area labelled the same area of the thalamic reticular nucleus. When injections were made into the gustatory thalamic reticular nucleus, there was labelling in the parvicelluar part of the thalamic posteromedial

ventral nucleus, suggesting that gustatory thalamic reticular nucleus receives innervation from cortex and thalamus, and projects back to thalamus Hayama, Hashimoto and Ogawa (1994).

1.2.3.6 Rostral thalamic reticular nucleus

Rather than representing connectivity with a sensory component of both thalamus and cortex, the rostral sector of the thalamic reticular nucleus has strong connections with cognitive, limbic, and motor areas. The rostral sector of the thalamic reticular nucleus does not have the same topographic organisation (from limbic and cognitive areas) as the caudal, sensory thalamic reticular nucleus. Projections to the rostral pole of the thalamic reticular nucleus focus can be divided into three areas: cognitive, limbic and motor.

Lozsádi (1994) first detailed limbic projections to the rostral sector of the thalamic reticular nucleus after injections into rat cingulate cortex. Unlike sensory sectors of the thalamic reticular nucleus there was no slab like organisation, with projections from the limbic cortex overlapping with projections from retrosplenial cortex. Retrosplenial cortex has strong connections with anterior thalamic nuclei, as does the thalamic reticular nucleus (Lozsádi, 1995). Neurons arising from the anterior thalamus project to the dorsal portion of the rostral thalamic reticular nucleus, which branch off toward the retrosplenial granular cortex (Gonzalo-Ruiz, Morte, & Leiberman, 1997). The dorsal portion of the rostral thalamic reticular nucleus reciprocates, projecting to the posterior, and medial divisions of the anterioventral thalamus, as well as to dorsolateral sector of the anterodorsal

thalamic nucleus (Gonzalo-Ruiz, & Lieberman, 1995). Retrosplenial cortex and anterior thalamic nuclei are implicated in memory, especially episodic and spatial memory (Wolff, Gibb, Dalrymple-Alford, 2006; Vann, Aggleton, & Maguire, 2009).

Further strengthening the view that the rostral sector of the thalamic reticular nucleus is involved in widespread cognition are the reciprocal connections between with mediodorsal thalamus. Rostral thalamic reticular nucleus projects to the mediodorsal thalamus in a topographical manner, with rostral projections arising from thalamic reticular nucleus innervating the rostral sector of mediodorsal thalamus (Groenewegen, 1988). The mediodorsal thalamus has strong reciprocal connections with prefrontal cortex, especially medial and orbital prefrontal cortex which is believed to be a foundation for a cognitive circuit (see Mitchell & Chakraborty, 2013 for a review of mediodorsal thalamus involvement).

The thalamic reticular nucleus is connected to the principle motor thalamic nuclei which comprise the ventral anterior and ventral lateral thalamic nuclei. Single neuron tracing has shown that motor thalamic neurons exiting the thalamus sends collaterals to the thalamic reticular nucleus (Kuramoto, Furuta, Nakamura, Unzai, Hioki, & Kaneko, 2009).

Stimulating motor cortex causes burst firing in the thalamic reticular nucleus which subsequently project to ventral anterior and ventral lateral thalamic nuclei, causes inhibitory postsynaptic potentials (Frigyesi, 1972). Additionally, there are indirect projections from the motor cortex en route to the ventral lateral thalamus. Rostral thalamic reticular nucleus receives topographical projections from the motor cortex, with a dorsoventral organisation in the thalamic reticular nucleus that corresponds to the caudorostral body representation that has been documented in

the motor cortex. Projections from motor cortex representing hindlimbs have been shown to be segregated topographically from those parts of the motor cortex that represent the forelimb and the head. Additionally, projections to the ventral lateral thalamus are topographic with connections from thalamic reticular nucleus and motor cortex within the ventral lateral thalamus terminating in the same area (Cicirata, Angaut, Serapide, and Panto, 1990).

1.3 The thalamic reticular nucleus and psychopathologies

1.3.1 The thalamic reticular nucleus and schizophrenia

One of the cornerstones of the cognitive issues seen in schizophrenic patients is cognitive flexibility, or a lack thereof. Cognitive flexibility is a somewhat ubiquitous term; used in various contexts, all with slightly different meanings/implications. Within the remit of "cognitive flexibility" is specific performance in tasks of attentional set-shifting. Attentional set-shifting refers to the ability to alter performance on a cognitive task in the face of changing task contingencies. In humans this is often studied using either the Wisconsin card sorting task or the intra-extradimensional set shift component of the CANTAB task. Both of these tasks measure the ability of an individual to acquire a rule, reverse it, as well as shift attention from one relevant dimension to another. The tasks are therefore sensitive to various deficits. It has been shown that schizophrenic individuals have significant impairments at the extradimensional shift stage. It is expected in all participants that more trials will be required to achieve criterion on the extradimensional stage, but schizophrenic patients demonstrate a rigidity that results in performance

significantly worse than controls (Pantelis, Barber, Barnes, Nelson, Owen, & Robbins, 1999; Jazbec, Pantelis, Robbins, Weickert, Weinberger, & Goldberg, 2007).

Because of the robust extradimensional shift deficit many studies using animal models of schizophrenia examine set shifting abilities as a form of validation. Being able to detect such deficits in an animal model is potentially indicative that they have mirrored at least some of the cognitive deficits seen in the disease. One pharmacological model of schizophrenia in rats is the phencyclidine model. It has been well established that phencyclidine can induce many of the symptoms of schizophrenia (Allen & Young, 1978; for a review see Morris, Cochran, & Pratt, 2005), and it is possible that part of this effect is mediated by alterations in thalamic reticular nucleus function.

Egerton, Reid, McKerchar, Morris, and Pratt (2005) examined attentional set-shifting performance in rats following acute administration of phencyclidine. Modified from the original Birrell and Brown (2000) protocol, they found that phencyclidine treated rats exhibited the characteristic extradimensional set shifting impairments, More specifically they took significantly more trials than controls to reach criterion at this stage, with no significant differences in performance across other stages. It was additionally found that there was a 20% reduction in parvalbumin mRNA expression in dorsal aspects of the thalamic reticular nucleus, with a significant negative correlation found between extradimensional shift performance and mRNA expression. Parvalbumin positive neurons have been shown to be critical for the synchronization of neural activity through regulation of gamma oscillation synchrony (Bartos, Vida, & Jonas, 2007). Furthermore, post mortem studies of schizophrenic patients and animal models of schizophrenia implicated

parvalbumin dysfunction in the cognitive symptoms of the disease (Beasley and Reynolds, 1997; Lewis, Curley, Glausier, and Volk, 2012).

Parvalbumin expression can be used as a marker as to the extent of damage occurring as a result of excitotoxic lesions of the thalamic reticular nucleus. Weese, Phillips & Brown (1999) used markers for parvalbumin following unilateral lesions of the thalamic reticular nucleus. Intact thalamic reticular nucleus neurons are easily identified using parvalbumin stains. Given the support for the view that parvalbumin positive interneurons are partially involved in the pathophysiology of schizophrenia, and the presence of parvalbumin in the thalamic reticular nucleus, it is fair to propose that the thalamic reticular nucleus may be involved in the cognitive aspects of the disease.

The thalamic reticular nucleus plays a pivotal role in the initiation and generation of sleep spindles that appear during the early stages of sleep. The thalamic reticular nucleus is therefore implicated in those disorders with an aberrant sleep component. For example, recent electrophysiological studies in humans have revealed marked differences in sleep spindles, generated by the thalamic reticular nucleus, between schizophrenic individuals and both healthy and psychiatric control samples. More specifically, it was shown by Ferrarelli, Peterson, Sarasso, Riedner, Murphy, Benca, Bria, Kalin, and Tononi (2010) that schizophrenic patients had whole night deficits in spindle power, slow and fast spindle amplitude, duration, number and integrated activity.

Additional research implicates the thalamic reticular nucleus in sensory gating – deficits in which are manifested in schizophrenia (Krause, Hoffmann, & Hajós, 2003). It is hypothesised that the sensory overload experienced by

schizophrenics reflects aberrant sensory gating, whereby irrelevant information is not adequately filtered.

1.3.2 Epilepsy

Much research into the role of the thalamic reticular nucleus focuses on its role in absence epilepsy. Thalamic nuclei are becoming a target for deep brain stimulation for intractable epilepsy (for a review see Mehranfard et al, 2015). Absence epilepsy is most common in children, and is characterised by sudden losses of consciousness and unresponsiveness accompanied by 3Hz spike-wave discharges (Aker et al, 2006).

Both in vivo and in vitro studies have concluded that both the thalamus and cortex are involved in the generation of spike and wave discharges. It is additionally believed that spike wave discharges, and sleep spindles involve the same corticothalamic network (Blumenfeld & McCormick, 2000). The thalamic reticular nucleus has been implicated in the generation of sleep spindles for decades, with Steriade, Domich, Oakson, and Deschenes (1987) showing that spindle related rhythms are maintained in the deafferented thalamic reticular nucleus of cats. Additionally, lesions of thalamic reticular nucleus using kainic acid have been shown to abolish spindle activity in thalamic neurons, suggesting that the thalamic reticular nucleus is critical for the generation of sleep spindles (Steriade, Deschenes, Domich & Mulle, 1985).

One of the most frequently used rodent models of absence epilepsy are the genetic absence epilepsy rats from Strasbourg. These rats show spontaneous spike and wave discharges (5-9Hz) during unresponsive and immobile states of

wakefulness (Pinault, Vergnes, & Marescaux, 2001). The same pattern of activity (5-9Hz oscillations) is also recorded in non-epileptic rats, suggesting that the thalamocortical system is involved not only in typical physiogical states of arousal, but also in states of hypersynchronous oscillations (Pinault, 2003).

Liu, Vergnes, Depaulis, and Marescaux (1991) showed that spike and wave discharges arising from the thalamic reticular nucleus can be suppressed by local microinjections of both the anti-epileptic drug gamma-vinyal-GABA, and muscimol in genetic absence epilepsy rats from Strasbourg.

Direct comparison of GABA+ve neurons in the thalamic reticular nucleus of Wistar rats with genetic absence epilepsy rats from Strasbourg (selected inbred strain of Wistars) further suggests a critical role for the area. GABA+ve and GABA-ve neurons were counted in rats at postnatal days 10, 20, 30 and 60. Counts using light-microscopical GABA immunohistochemistry revealed an increase in both neurons from postnatal day 10 to 20 in both groups. However, while Wistars showed no significant decline in cell density from postnatal days 30 to 60, the genetic absence epilepsy rats from Strasbourg showed a significant reduction in density of both GABA+ve and GABA-eve cells. There were no morphological differences in the remaining cells, leading the researchers to propose that the abnormal development of thalamic reticular nucleus could account for the spike and wave discharges seen during the epileptic episodes in the genetic absence epilepsy rats from Strasbourg (Çavdar, Bay, Kirazlı, Çakmak, & Onat, 2013). This is consistent with Vergnes, Marescaux, Depaulis, Micheletti, and Warter (1986) who showed that inbred strains of Wistars presenting with spontaneous electrological and clinical symptoms of petit mal-like seizures did not manifest their first EEG spike and wave discharges until

postnatal day 40 and onwards. Furthermore, their number and duration was shown to increase with age, thus suggesting there is a developmental component that could relate to abnormal thalamic reticular nucleus development.

1.3.3 Parkinson's Disease

Parkinson's disease is a complex and progressive neurodegenerative disorder. The disease is often characterised based upon motor symptoms such as abnormal posture and involuntary tremors, but there are also a myriad of non-motor symptoms including sleep and autonomic disturbances, mood changes, and cognitive decline (Garcia-Ruiz, Chaudhuri, Martinez-Martin, 2014). The main aim of many clinical trials is still to ameliorate the physical symptomology of the disorder, despite researchers acknowledging that it is the non-motor symptoms of the disease that are most correlated with both quality of life and disease progression (Global Parkinson's Disease Survey Steering Committee. 2002; Chaudhuri,Healy,& Schapira, 2006; Chaudhuri, & Schapira, 2009).

Up to 80% of patients with Parkinson's disease go on to develop Parkinson's disease dementia (Halliday, Hely, Reid, & Morris, 2008). However, before the onset of Parkinson's disease dementia, patients often show a marked decline in cognitive function such as attention and memory deficits (Foltynie, Brayne, Robbins, & Barker, 2004; Aarsland, Brønnick, Larsen, Tysnes, Alves, & Norwegian ParkWest Study Group, 2009). Decline in attention was shown by Lawson and colleagues (2016) to be the biggest predictor of quality of life during disease progression in those patients with Parkinson's disease who were also exhibiting signs of mild cognitive impairment.

Frontal dopamine levels in Parkinson's patients has been shown to be correlated with performance in the attentional set shifting task, with lower levels of the neurotransmitter associated with lower cognitive flexibility and therefore more trials to criterion at the extradimensional stage (Fallon, Smulders, Esselink, van de Warrenburg, Bloem, & Cools, 2015).

Given that one of the main anatomical markers of Parkinson's disease is degeneration of the substantia nigra pars compacta (Forno, 1996; McNaught, Belizaire, Jenner, Olanow, & Isacson, 2002), and this area has been shown to be connected to the thalamic reticular nucleus (Anaya-Martinez, Martinez-Marcos, Martinez-Fong, Aceves, & Erlij, 2006; Freeman, Ciliax, Bakay, Daley, Miller, Keating, Levey, and Rye, 2001) - it is possible that some of the cognitive symptoms of the disease may be due to aberrant innervation of the thalamic reticular nucleus. Attentional set shifting has been shown to be prefrontal dependent (Birrell & Brown, 2000; McAlonan & Brown, 2003), and we know from previous research that the thalamic reticular nucleus has strong connections with the prefrontal cortex (Pratt & Morris, 2015). Although speculation at this point, it is possible that the perturbations in thalamic reticular nucleus activity could account for some of the non-motor symptoms observed during the progression of the disease. It would be of interest to study the anatomy and function of the thalamic reticular nucleus in established rodent models of Parkinson's disease, as this could shed some light as to the role, if any, the thalamic reticular nucleus plays in non-motor symptoms.

1.4 The thalamic reticular nucleus and attention

1.4.1 Crick's Searchlight Hypothesis

Although research interest in the role of the thalamic reticular nucleus in attention has increased somewhat in recent years, it is a relatively long standing idea. Incoming sensory information reaches cortex through the thalamus, and in the face of this sensory bombardment animals are still able to selectively attend to salient stimuli that could signal danger or valuable opportunity. The incoming sensory information must be filtered, so as to facilitate the relay of information considered behaviourally pertinent, while marginalising irrelevant input. It has been traditionally stated that the thalamus is the sensory gateway to the cortex, while Crick (1984) proposed that the thalamic reticular nucleus "might be considered the guardian of that gateway" (p. 4587).

Crick (1984) proposed that the thalamic reticular nucleus was central to his Searchlight Hypothesis. He proposed that there was an internal attentional searchlight that could monitor cortical and thalamic activity in order to determine a locus of 'interest'. Once the key environmental stimulus had been identified, it would be able to exploit its connectivity with the aforementioned regions to intensify thalamic input to corresponding cortical regions. Increasing the intensity of thalamic firing would therefore enhance the processing of the seemingly relevant stimuli while marginalizing the processing of other stimuli. As we interact with a dynamic world, the nucleus must then be able to re-allocate its focus to the next place that demands attention.

1.4.2 Was Crick correct?

The main evidence that Crick (1984) used to support his argument, was the extensive inhibitory collaterals arising from the thalamic reticular nucleus that were able to tap into, and select, small regions of the thalamo-cortical map that were most active. These inhibitory connections then produce small bursts of activity in thalamic cells, followed by a corresponding cessation in activity during which time focus could be directed to another more active area (Crick, 1984). It appears that the mechanism that Crick is describing, to some extent, is lateral inhibition. Reducing the activity of certain thalamic neurons permits greater allocation of resources to the processing of information from other thalamic regions. In essence, this mechanism could be used to explain the pattern of results when measuring presented by McAlonan, Brown and Bowman (2000) regarding thalamic reticular nucleus activity in the Kamin blocking task.

Using operant boxes and food reward, rats were divided into three groups: for one group a tone was the conditioned stimulus and a light was the blocked stimulus, for another the light was the conditioned stimulus and the tone was the blocked stimulus, and finally one group received a compound stimulus of tone and light. Immediately after the final testing session rats were transcardially perfused and their brains were processed for the presence of Fos protein. The expression of the protein product of c-*fos* is used as a marker of neural activity in studies attempting to map the function of the nervous system (Zangenehpour & Chaudhuri, 2002).

Immunohistochemical analysis showed significantly greater Fos-positive labelled neurons in the sector of the thalamic reticular nucleus associated with the

conditioned stimulus as compared to the blocked stimulus – rats conditioned to the tone showed greater auditory thalamic reticular nucleus activation, and those conditioned to the light showed greater visual thalamic reticular nucleus activation. Animals conditioned to the compound showed equal activation across auditory and visual thalamic reticular nucleus (McAlonan, Brown & Bowman, 2000).

The lateral inhibition that could explain the McAlonan, Brown and Bowman (2000) data is inhibition originating from thalamic reticular nucleus. There is an extensive network of inhibitory interconnectivity across subsectors of the thalamic reticular nucleus, as well as sectors representing the same sensory dimension in thalamus (Zhang & Jones, 2004). This lateral inhibition serves to alter the pattern of inhibition projected onto thalamic subsectors. It is through this mechanism that the relay of relevant or predictive information is processed at the expense of irrelevant information. There is of course an issue with placing too much functional significance on data obtained from a Fos study, as although we know that the thalamic reticular nucleus was active during the blocking task, we cannot extrapolate the true thalamic reticular nucleus function. Without a follow up study where function can be altered, such as through lesions, we cannot make too many inferences about the true contribution of the thalamic reticular nucleus to attention within blocking conditions. However, one could speculate that the mechanism involved could indeed be lateral inhibition.

Such actions are cortically driven, as demonstrated by Montero (2000). Rats who received unilateral ibotenic acid lesions of layer 6 of primary visual cortex (without retrograde degeneration of lateral geniculate nucleus) showed reduced Fos expression in the corresponding visual sector of the thalamic reticular nucleus when

exploring a novel environment. The number of Fos positive neurons in the visual sector of the unlesioned side showed a pattern of activation consistent with the view that the thalamic reticular nucleus was involved in attending to the new visual aspects of the complex environment. In contrast, the visual sector of the lesioned side showed a significant reduction in positive neurons, thus suggesting that thalamic reticular nucleus involvement in attention is cortically driven (Montero, 2000).

1.4.3 Animal studies of the thalamic reticular nucleus

Despite issues with Crick's hypothesis, it is clear that the thalamic reticular nucleus is involved in attention. The size, shape and location of the thalamic reticular nucleus make it difficult to lesion. A knock on effect of this, is that behavioural evidence examining perturbations to thalamic reticular nucleus function is somewhat limited. However, the data available strongly implicates the thalamic reticular nucleus in attention.

Weese, Phillips, and Brown (1999) showed that cell body lesions of the thalamic reticular nucleus abolish the validity effect in the Posner task. Rats were trained to respond, using a nose poke, to a bright light that would appear immediately to the right or left of the central aperture. Preceding this event, a dim cue light would appear either on the right or left. In 50% of the trials the dim light would appear on the same side as the target stimulus – a valid cue – and 50% of the time the target light would appear on the opposite side, making it an invalid cue. Prior to surgery reaction time was faster to target stimuli in the valid cue trials as compared to the invalid cue trials, which can be attributed to both a benefit of

directing attention towards the target location, as well as a cost for misdirecting attention. Following unilateral ibotenate lesions of thalamic reticular nucleus the validity effect was abolished; reaction time to validly and invalidly cued targets to the side contralateral to the lesion was no longer different. The benefit of processing the information presented by the cue and subsequently using it to guide attention was abolished – the animals forgoing the use of the top-down information, using only the target information and not the preceding guiding cue.

In freely behaving animals, Montero (1997) studied thalamic reticular nucleus activity when exploring a novel environment. When placed in a new environment rats adopt exploratory behaviour, directing attention towards key features of the location. A selective increase in Fos positive neurons was found in the visual sector of the thalamic reticular nucleus following exposure to the novel environment. Functionally blind rats who explored the same novel complex environment showed a selective increase in Fos positive neurons in the somatic sector of the thalamic reticular nucleus. Not surprisingly, there were few fos positive neurons in the visual sector in these animals. Sector specific activation of the thalamic reticular nucleus pertaining to the predominant sensory channel used could implicate the thalamic reticular nucleus in optimizing transmission of behaviourally pertinent sensory information through the thalamocortical circuit.

Petrof and Brown (2010) measured c-fos expression in the visual and somatosensory sectors of the thalamic reticular nucleus in a task of discrimination learning. Animals were trained to dig in bowls based solely upon either their visual (bowl colour: black or white) or tactile (bowl texture: rough or smooth) characteristics. Rats were first trained to dig in bowls that differed either by their

texture or by their colour (simple discriminations). Following stages introduced the second dimension, resulting in compound discriminations. Reversal stages were added, so that the animals were equally exposed to all stimuli. Immunocytochemical analysis revealed greater Fos activation in the visual sector of the thalamic reticular nucleus of those rats that performed the visual discrimination version of the task one hour prior to perfusion. Interestingly, there was no corresponding increased number of Fos positive neurons in the somatosensory sector of the thalamic reticular nucleus in those rats that performed the tactile discrimination. Fos positive neurons in lateral geniculate nucleus were similar across both groups, demonstrating that increased visual thalamic reticular nucleus in the visual task rats was not merely a function of visual stimulation. Rather, the increased visual thalamic reticular nucleus in the visual discrimination task is seen to represent visual selective attention. The rats were trained under conditions with low light levels, which made the visual discrimination quite demanding. In contrast, the low luminance levels should not have been an issue for the rats performing the tactile task and it is possible therefore that the attentional demands were not completely equal. If the tactile discrimination was not sufficiently difficult – perhaps the differentiation between rough vs smooth was too distinct –this could explain why there was no increased Fos activation in somatosensory thalamic reticular nucleus.

Evidence additionally suggests that the thalamic reticular nucleus is involved in sensory gating. Often studied in the auditory domain, sensory gating is the attenuation of a neural response to a presented stimulus if it is preceded by a warning stimulus (Jessen, Kucharski, Fries, Papassotiropoulos, Hoenig, Maier, & Heun, 2001). Traditionally it is studied using paired click paradigms in which two

identical tones are presented in pairs, with an interstimulus interval of approximately 500ms. It has been reliably shown that there is a significant reduction in neural responses of the P50-N100-P200 complex to the second stimulus of the pair (Rentzsch, Jockers-Scherübl, Boutros, & Gallinat, 2008). This paradigm has strong translational value, and is often used to study the efficacy of antipsychotic medications in rodents in early stages of neuroscience and medical research (Swerdlow, Braff, Taaid, & Geyer, 1994; Potter, Summerfelt, Gold, & Buchanan, 2006).

Sensory gating can be considered to indicate the presence of inhibitory mechanisms that serve to protect the integrity of processing by marginalizing the transmission of irrelevant information (Popov Jordanov, Rockstroh, Elbert, Merzenich., & Miller, 2011). Attention, by its very nature, requires filtering of sensory information and the inhibition of processing irrelevant information. Therefore, when measured in the thalamic reticular nucleus, sensory gating may reflect attentional mechanisms – a reduced response to the second tone reflecting reduced allocation of attentional resources. Differences in neural responses could reflect early allocation of attention, with the function of protecting higher up cognitive processing from irrelevant information. Krause, Hoffmann, and Hajos (2003) examined auditory sensory gating in the thalamic reticular nucleus of anaesthetized rats. Single unit recordings of thalamic reticular nucleus neurons revealed a greater number of spikes in response to the first of the two tones, demonstrating sensory gating. The reduced response to the second tone reflects normal auditory processing. Administration of D-amphetamine disrupted sensory gating in the thalamic reticular nucleus, with a greater number of responses

recorded for the second test stimulus. Additionally, the firing pattern changed from burst to single spike firing, resulting in a more irregular firing pattern. Both changes evoked by D-amphetamine were reversed by administration of haloperidol. Impairments in thalamic reticular nucleus firing rats could have a significant impact on thalamic auditory processing given connectivity with the medial geniculate nucleus. Although this cannot be directly determined by these results, it is conceivable that changes in sensory gating in the thalamic reticular nucleus could account for sensory overload often described in schizophrenic patients.

1.5 Aims of this thesis

The primary goal of this thesis was to obtain further behavioural evidence of the role of the thalamic reticular nucleus in attention. It is believed that the thalamic reticular nucleus plays a significant role in regulating states of consciousness and attention – aberrant activity of this region causing many of the cognitive deficits seen in neurological conditions such as Parkinson's disease, Schizophrenia and epilepsy. Despite the importance attributed to this region and the need to understand the exact mechanisms of typical and atypical attention performance, there is a lack of behavioural research focused on the thalamic reticular nucleus. The void in the literature is due in part to the location of the thalamic reticular nucleus. As a thin layer of neurons arranged around the dorsal thalamus in a shell shaped formation, it is very difficult to produce focal lesions. Despite this, it is still critical that we characterize the exact contributions of the thalamic reticular nucleus if we are to better understand attention deficits manifested in neurological disorders.

By combining the use of excitotoxic and dopamine depleting lesions with behavioural tests of attention, the aim of the present thesis was to extend knowledge of the functional role of the thalamic reticular nucleus in attention. In chapter 3, we examine the role of the thalamic reticular nucleus in blocking out distracting stimuli in a cross modal distraction task. More specifically, in an operant task of visuospatial attention, we sought to determine whether the thalamic reticular nucleus actively blocks transmission of distracting, and irrelevant, sensory stimuli that are in a different domain to that they are attending to. By using excitotoxic lesions in the visual thalamic reticular nucleus it was hypothesised that rats will make more errors in trials with an auditory distractor because they cannot adequately block processing the distractor.

In Chapter 4, the effect of cell body lesions of the thalamic reticular nucleus on performance in the 7 stage attentional set shifting task is examined. In addition rats perform a simple visual discrimination task following surgery. The thalamic reticular nucleus has widespread connectivity with prefrontal areas, and it has been shown that optimal performance in the task is prefrontal dependent. As the thalamic reticular nucleus is implicated in determining which incoming sensory information reaches cortex, it was hypothesised that lesions of the thalamic reticular nucleus would impair performance.

In Chapter 5, the role of the thalamic reticular nucleus in attentional set shifting is examined by comparing lesions of the rostral, or cognitive, thalamic reticular nucleus with those of mediodorsal thalamus lesions. Mediodorsal thalamus lesions have been shown to impair performance on tasks that are known to be prefrontal dependent, and it is hypothesised that in tasks with an attentional

component this may be due, in part, to connectivity with the rostral thalamic reticular nucleus.

In chapter 6, 6-hydroxydopamine injections into the thalamic reticular nucleus were used to deplete dopaminergic innervation to the thalamic reticular nucleus. We then compare behaviour in a visuospatial attention task compared before and after surgery. The task is a top down task of attention, in which animals can predict target onset location across a variable foreperiod. Given the involvement of dopamine in the re-orientation of attention, it was hypothesised that depleting dopamine in attention will impair the shifting of attention throughout the variable foreperiod.

This thesis details the use of surgical and behavioural techniques to investigate the role of both the caudal and more sensory, and rostral and more cognitive, sectors of the thalamic reticular nucleus to attentional processes.

General Methods and Materials

2. 1 Subjects

All animals used were male Lister-hooded rats (Charles River, UK). All animals were experimentally naïve. All experiments were conducted under licensed authority, in accordance with the UK Animals (Scientific Procedures) Act 1986.

Animals were housed in groups of three in home cages that measured 10" x 14.5" x 22". A clear plastic tube was suspended in the ceiling of each cage for the rats to climb and sit/sleep in. Chewing materials (wooden stick and cardboard 'tent') were provided in each cage in addition to paper bedding. Each room was maintained on a 12 hour light/dark cycle (lights on at 7am). The temperature of the room was maintained at between 18.5 and 23°C at all times with a humidity of 55±10%.

Water was provided ad libitum, and rats were placed on a diet of 15-20g of standard laboratory chow each day. Controlled access to food was used to keep rats at a healthy weight, and to increase motivation to work for small food rewards. Rats were weighed once a week to ensure a healthy weight and growth.

2.2 Apparatus

2.2.1 Operant Chambers

Figure 2.1 depicts the nine-hole operant chamber used in Chapter 6. Of the nine poke holes available, only the central three were used – the remaining six were blocked with covers. At the back of each hole was a white LED for illumination, and a photoelectric cell at the entrance to detect a break in a vertical infrared beam when the rat pokes its nose into the hole. A food dispenser to the side of the chamber

delivered a single 45mg pellet (Testdiet, Richmond, IN, USA) to a food hopper on the chamber wall opposite the nine-hole array. The hopper contained a light within, which was used to signal the availability of food or the requirement to push the hinged vertical panel (for example, to initiate a trial after an error). A loudspeaker was contained within the box to deliver auditory stimuli, and a house-light was illuminated during the session, except during periods of 'timeout' following errors. The testing chamber was contained within a sound-attenuating outer box with a fan to provide both ventilation and also a background level of white noise.

The operant chambers used in Chapter 3 were similar, but had additional stimulus lights above and below the poke-holes.

Figure 2.1. Example of one of the nine hole operant boxes.

2.2.2 Set-shifting apparatus

Figure 2.2 depicts an example of the attentional set shifting apparatus. Boxes were constructed in the School of Psychology workshop using large home-cages (10” x 14.5” x 22”), with the addition of a clear acrylic lid. A large divider panel could be inserted to close off one-third of the length of the box, to form a ‘holding area’ (the larger side of the partition) and two areas, with a central divider between them, in the smaller part of the box where the digging bowls were placed. The holding area of the box contained sawdust and a water bowl. The digging bowls themselves were round ceramic pet food bowls, which were 4cm deep and 7cm in diameter. The digging bowls could be filled with a large variety of scented digging media (see details in the methodology). For the visual discrimination task, the outer surfaces of the bowls were painted with horizontal or vertical black and white stripes (see figure 2.3)

Figure 2.2. Example of one of the attentional set shifting testing boxes and example stimuli.

Figure 2.3. Examples of the striped digging bowls used in the visual discrimination

2.3 Testing

The detailed description of each task protocol (including training and trial types) is provided in each individual chapter.

2.3.1 Justification for tasks chosen

The cross-modal distraction task was chosen as it allowed an experimental follow up to the McAlonan, Brown, & Bowman paper which examined Kamin blocking. It was posited that lateral inhibition was the key mechanism observed in the experiment. The cross modal distraction task is thought to rely on the mechanism of lateral inhibition by requiring the animal to block the processing of information pertaining to the distractors in order to dedicate attention to the relevant stimulus and response required.

The spatiotemporal serial reaction time task provides information about movement time, but more importantly information about the orientation, disengagement, and re-orientation of attention. The effects of manipulations can be compared across various dimensions of attentional engagement/disengagement. Due to the requirement to simply follow the location of the stimulus and respond with a nose poke, it is fairly simple for the animals to learn, and therefore has a high rate of completion.

The attentional set shifting task was chosen for use in this thesis due to its sensitivity to both attentional and reversal deficits. Its use amongst various animal models, and its analog in human studies, means that well established patterns of behaviour across neurological conditions have been established. With this in mind, one can then compare the effects of lesions or manipulations on performance, and

potentially extrapolate whether such manipulations could represent involvement in any neurological disorders. Digging is a natural behaviour for rats, and it is therefore easy to train animals in the task. A single day is sufficient to complete an entire run of the task, making it an ideal task to test a large number of animals.

2.3.2 Cross-modal distraction task (Chapter 3)

In the cross modal distraction task, animals were required to perform a two-alternative forced choice reaction time task. After making a central nose poke, a light appeared either above or below the rat, the response required was counterbalanced so that for half of the animals a light above required a nose poke immediately to the left of the central hole, and a light below required a nose poke immediately to the right of the central hole.

2.3.3 Attentional set shifting Task (Chapters 4 and 5)

The attentional set shifting task was first described by Birrell and Brown (2000), with the testing phase comprising 7 distinct stages. These details will describe one example of the task where digging medium is the first relevant stimulus. Initial rewarded dimension, and rewarded exemplar were counterbalanced across animals (See table 2.1). Rats had to get six trials in a row correct to progress between stages. The stages were as follows:

Stage 1 (Simple Discrimination) – In this stage only one dimension was introduced, and the rat has to find the reward in the sand (no reward in the grit).

Stage 2 (Compound discrimination) – A second, irrelevant dimension was introduced (odour), but the relevant dimension was still media and sand was still rewarded.

Stage 3 (Reversal 1) – Previously correct and incorrect responses were reversed so that the reward was placed in the grit, not sand.

Stage 4 (Intradimensional shift) – All new exemplars were introduced, but the reward was still in the digging media (Coarse sawdust, not fine sawdust).

Stage 5 (Reversal 2) - Previously correct and incorrect responses were reversed so that the reward was placed in the fine sawdust, not coarse sawdust.

Stage 6 (Extradimensional shift) – A final set of exemplars were introduced, and the previously irrelevant dimension became relevant and therefore rewarded. The rat had to learn to find the reward based on the odour of the bowls, not the digging media (reward in cinnamon, not ginger).

Stage 7 (Reversal 3) - Previously correct and incorrect responses were reversed so that the reward was placed in the ginger, not the cinnamon.

Stage	Discrminanda	Paired with
Simple discrimination	M3(Sand), not M4 (Grit)	No pairing
Compound discrimination	M3(Sand), not M4 (Grit)	O3 (Sage), or O4 (Paprika)
Reversal 1	M4 (Grit), not M4 (Sand)	O3 (Sage), or O4 (Paprika)
Intradimensional discrimination	M5 (Coarse sawdust), not M6 (Fine sawdust)	O5 (Turmeric), or O6 (Clove)
Reversal 2	M6 (Fine sawdust), not M5 (Coarse sawdust)	O5 (Turmeric), or O6 (Clove)
Extradimensional discrimination	O1 (Cinnamon), not O2 (Ginger)	M1 (Coarse tea), or M2 (Fine tea)
Reversal 3	O2 (Ginger), not O1 (Cinnamon)	M1 (Coarse tea), or M2 (Fine tea)

Table 2.1. An example of the stages and stimuli used in the 7-stage attentional set shifting task. The stages were always run in the same order, with the correct stimuli counterbalanced across rats.

2.3.4 Spatiotemporal target probability signal reaction time task (Chapter 6)

The spatiotemporal target probability signal reaction time task was a two alternative forced choice reaction time task. After making a central nose poke, a light appeared immediately to the right or left of the central hole. The rat was required to withdraw its nose from the central hole and subsequently poke in the hole where the flanker light appeared. The spatial probability of target location was varied as a function of foreperiod length, with the target being significantly more likely to appear at the left at earlier foreperiods, and right at later foreperiods. The right:left ratio of target onset was 8:92 at 200ms; 33:66 at 300ms; 50:50 at 400ms; 66:33 at 5ooms and 92:8 at 600ms.

2.4 Surgery

The experimental approach taken in this thesis was to effect lesions, using toxins, of the thalamic reticular nucleus. As described above (Chapter 1, page 19), the thalamic reticular nucleus is a collection of GABA-ergic neurons that receive dopaminergic input from the ventral tegmentum. Therefore, 6-hydroxydopamine was used to remove the dopaminergic input and ibotenic acid was used to cause cell-body lesions.

2.4.1Toxins

2.4.1.1 6-hydroxydopamine (6OHDA).

6-hydroxydopamine is a compound used to produce lesions only in monoamine neurons. Selective uptake by catecholamine neurons means it is an ideal tool to study the anatomy of, and functional role for, dopaminergic and noradrenergic neurons (Ungerstedt, 1968).

The formation of reactive oxygen species through the autoxidation of 6OHDA is now widely accepted to be the mechanism through which the compound is neurotoxic. Oxidization of 6OHDA within the cell results in hydrogen peroxide forming is belived to be partially responsible for the nerve degeneration following toxin infusion (Saccs and Jonsson, 1975). Additionally the production of reactive oxygen species following extracellular oxidation leads to further oxidative stress. It has been proposed that the sensitivity of dopaminergic neurons to oxidative stress could explain the toxins selective effect on this class of neurons (Berretta, Freestone, Guatteo , de Castro, Geracitano, Bernardi, Mercuri, & Lipski, 2005; Hanrott,

Gudmunsem, O'Neill, & Wonnacott, 2006; Rodriguez-Pallares, Parga, Muñoz, Rey, Guerra, & Labandeira-Garcia, 2007).

2.4.1.2 Ibotenic acid

Ibotenic acid, also referred to as ibotenate, is a compound found to be naturally occuring in the mushroom *Amanita muscaria.* Structural similarities to the neurotransmitter glutamate allow it to serve as a nonselective glutamate agonist for neuroscientific research. Prolonged pre- and post-synaptic depolarisation at the site of infusion leads to cell death. Comparing the action of ibotenic acid with an alternative excitoxin, kainic acid, has shown that the former is able to produce more selective lesions (Schwarcz, Hökfelt, Fuxe, Jonsson, Goldstein, & Terenius, 1979).

2.4.2 General Surgical Procedure

Isoflurane anaesthesia was used. The animal was placed in an induction chamber and anaesthesia was induced with a mix of 5% isofluorane in oxygen. Once anaesthetised the rat's scalp was shaved, they were injected subcutaneously with 0.05ml carprieve, a non-steroidal anti-inflamatory, and were placed in the stereotaxic frame (Kopf, Tujunga, CA) using atraumatic ear bars with the tooth bar set at -3.3mm. The level of isofluorane was reduced gradually to 2-3% for the remaining time of the surgery. A midline incision was made and the tissue was retracted and held in place. Once the coordinates of the bregma and midline were found, a burr hole was made using an electric dental drill (coordinates taken from Paxinos & Watson, 1986).

The toxin was delivered by hamilton syringe (chapters 3 and 6) or pulled glass pippette (chapters 4 and 5), lowered to the relevant coordinates. Infusion time varied by volume and toxin type (see individual chapters).

Following the infusion of the toxin and the necessary waiting time, the incision in the scalp was thoroughly cleaned with saline and closed with sterilised metal wound clips. The closed wound was then cleaned with alcohol. Rats were then removed from the stereotaxic frame and placed in cages, lined with paper, with heating pads beneath. Once the animal could self-correct posture when placed on their side, they were removed from the recovery area and placed in cages in their home room. They were single housed for 7 days, and given wet mash (a mixture of ground lab chow and water) for 2 days following surgery. Rats were given at least 4 days to recover after surgery before testing recommenced.

After 7 days of being single housed, rats were returned to group housing with their previous cagemates. All reintroductions were successful, requiring only an hour of observation to ensure that no dominance displays resulted in injury.

Following initial recovery from the effects of surgery, there were no observable differences between lesion and control animals in general wellbeing or behaviour. All animals remained amenable to handling, maintained their weight, and showed regular sleeping patterns.

2.4.2.1Ibotenic acid.

The procedure as described above was followed, with the addition of an injection of 0.25ml diazepam to reduce the likelihood of injury as the excitotoxin was in effect. The excitotoxin can often cause repetitive behaviour including grinding of the teeth. The position often adopted by rats following surgery meant the animals

were at risk of injuring their own paws. This was mitigated by the administration of diazepam. As a further measure to minimise the self-inflicted injury plastic collars were used for several hours after surgery until the behaviour dissipated.

2.4.2.2 6-hydroxydopamine.

The general surgical method was followed, with the additional injection of desipramine, administered at least 20 minutes before toxin infusion. The injection was given at the same time as the carprieve injection. Desipramine is a noradrenergic reuptake inhibitor and acts to preserve the integrity of the noradrenergic system when using the toxin 6-hydroxydopamine.

2.5 Immunohistochemistry

Assistance with transcardial perfusion was provided by Dr David S. Tait. All other immunohistochemical procedures were completed without assistance.

2.5.1Transcardial perfusion

Rats were given an intraperitoneal injection of 0.8ml pentobarbitone. Once reflexes were no longer present they were perfused with 0.1M phosphate buffered saline for 30 seconds followed by 4% paraformaldehyde in phosphate buffer for 20 minutes. Brains were removed and placed in a 20% sucrose solution overnight before being processed.

2.5.2 Staining

A freezing microtome was used to cut 50μm thick coronal sections and placed in 0.1M phosphate buffer; every fourth section was stained. When

necessary, sections were kept in tubes in the freezer in ethylene glycol until ready to be stained.

2.5.2.1 Ibotenic acid staining.

Ibotenic acid lesions were visualized by staining the tissue for cresyl violet (to see cell bodies), NeuN (specific to neurons), and parvalbumin.

When tissue was removed from the freezer, all sections were washed three times (3 x 2 minutes) in phosphate buffered saline, and were subsequently placed in blocking solution (79% PBS, 20% goat serum and 1% of ten percent triton) and left for one hour on the rotatest shaker. The tissue was washed again (2 minutes) before being placed in antibody diluting solution (98% PBS, 1% goat serum, 1% of ten percent triton). The antibody diluting solution (ADS) contained the primary antibody, anti-NeuN (1:4000). The tissue was incubated in the primary solution on the shaker overnight. The tissue was washed five times in PBS (5 x 2 minutes) and subsequently placed in mouse IgG (Vector Laboratories Ltd) in ADS (5 μl/ml ADS) on the shaker for one hour. The tissue was then washed a further five times in PBS (5 x 2 minutes), and placed in the AB-complex (Vector laboratories) for one hour. Both A and B reagents were prepared at 10μl/ml ADS. Tissue was washed in PBS five times (5 x 2 minutes) and placed in in Diaminobenzidine solution (Sigma chemicals; Diaminobenzidine tablets in distilled water). The sections were left in the diaminobenzidine solution on the shaker until the desired stain was achieved (<10 minutes). The sections were washed for a final time (4 x 2 minutes) and then mounted onto glass slides. The slides were then placed in Xylene for several minutes and subsequently coverslipped using DPX glue to secure the slides.

2.5.2.2 Tyrosine hydroxylase.

Dopamine-depleting lesions were visualized by staining for tyrosine hydroxylase, which activity indicates the presence of functioning dopamine neurons. Sections were washed three times (3 x 2 minutes) in phosphate buffered saline (PBS), and subsequently placed in blocking solution (79% PBS, 20% goat serum and 1% of 10% triton) and left on the shaker for one hour. The tissue was washed again (2 minutes) in PBS and placed in antibody diluting solution (98% PBS, 1% goat serum, 1% of 10% triton). The antibody diluting solution (ADS) contained the primary antibody, Anti-TH at a concentration of 0.32µl/ml. The tissue was left in the primary antibody solution on the shaker overnight. Sections were subsequently washed five times (5 x 2 minutes) in PBS before being placed in mouse IgG (Vector Laboratories Ltd) in ADS (5 µl/ml ADS) on the shaker for one hour. The tissue was then washed in PBS five times (5 x 2 minutes), and placed in the AB –complex (Vector laboratories) for 1 hour. The A and B reagents were prepared at 10µl/ml ADS each. Sections were washed in PBS five times (5 x 2 minutes), and placed in Diaminobenzidine solution (Sigma chemicals; Diaminobenzidine tablets in distilled water) and placed on the shaker until the desired colour of sections was achieved (<10 minutes). The tissue was then washed four times (4 x 2 minutes) in PBS and mounted onto glass slides. Slides were then placed in Xylene for several minutes, and subsequently cover slipped using DPX adhesive to secure to slides.

2.6 Exclusions

At times, it may become necessary to remove animals from the final analysis. Although not ideal, there are several valid reasons to exclude animals from the final

analysis of an experiment. Reasons include a failure to adequately train or learn task parameters (often failing to reach a preimposed criterion of percentage correct responses), a failed manipulation such as a lesion in the wrong region (identified by immunohistochemistry), or death before the cessation of the experiment. (See Table 2.2 for a summary of animals omitted from the experiments in this thesis).

Chapter	Number of animals omitted	Reason(s) for omission
3	1	• Failure to recover from surgery
4	2	• Audiogenic seizures • Failure to complete all aspects of task
5	1	• Failure to recover from surgery
6	10	• Lost during surgery • Failure to recover from surgery • Audiogenic seizures • Failure to complete all aspects of the task

Table 2.2. A summary of the reasons for animal omission from analysis by experimental chapter.

Lesions of visual thalamic reticular nucleus does not increase the distractibility of auditory tones presented during a two alternative forced choice reaction time task

Abstract

It has been previously established that attention within a modality is modulated by the thalamic reticular nucleus, with lesions of the thalamic reticular nucleus impairing the shifting of visuospatial attention (Weese, Phillips, & Brown, 1999). Sector specific activation of the thalamic reticular nucleus is also associated with attention directed to one sensory dimension (McAlonan, Brown, & Bowman, 2000; Petrof & Brown, 2010). It is believed that the mechanism through which the thalamic reticular nucleus enhances the processing of behaviourally relevant information at the expense of superfluous stimuli is through lateral inhibition.

To date it has not been established whether the thalamic reticular nucleus is able to block the processing of irrelevant stimuli in a sensory dimension different to that of the relevant stimulus. Rats were trained to perform a visual discrimination task, with half of the trials having an irrelevant auditory stimulus presented alongside the relevant visual stimulus. One group of rats received bilateral visual thalamic reticular nucleus lesions using ibotenic acid, with a second group receiving sham lesions.

Before surgery it was found that the auditory stimuli were sufficiently distracting, with distractor trials resulting in a significantly lower percent correct score than non-distractor trials. However, no effect of the lesion was found – with no change in the percentage correct during distractor trials following surgery. These results suggest that the thalamic reticular nucleus is not involved in blocking of cross-modal distractors. Results are discussed in regards to the difficulty of the task, and potential avenues for paradigm development.

3.1 Introduction

The recruitment of relevant sensory sectors of the thalamic reticular nucleus during tasks of attention has been established through a series of Fos experiments. The expression of the protein product of c-*fos* can be used as a marker of neural activity in studies attempting to map the function of the nervous system (Zangenehpour & Chaudhuri, 2002). Montero (1997) showed selective activation of visual thalamic reticular nucleus in rats exploring a novel, complex environment. Visual cues were the predominant source of sensory information for the rats, and this was reflected in enhanced fos expression in visual thalamic reticular nucleus. Similarly, functionally blind rats showed enhanced fos expression in somatosensory thalamic reticular nucleus when exploring the same environment. In the absence of visual information the rats relied upon tactile information gathered from their whiskers, as indicated by increased expression in the cortical whiskers barrel field (Montero, 1997).

Further behavioural evidence examined thalamic reticular nucleus involvement in Kamin blocking (McAlonan, Brown, & Bowman, 2000). Kamin blocking uses the tenets of classical conditioning, in which a stimulus that reliably predicts reward is preferentially processed over a secondary stimulus that has no independent predictive value. As the secondary stimulus, presented simultaneously with the predictive stimulus, does not relay any additional information pertinent to the onset of the reward its processing is marginalised. In this paradigm the stimulus that predicts reward and is therefore attended to is known as the conditioned

stimulus, while the secondary stimulus is known as the blocked stimulus (See Campbell, Church, & Kamin, 1969).

McAlonan, Brown, and Bowman (2000) divided rats into three experiment groups: for one group a tone was the conditioned stimulus and a light was the blocked stimulus, for another the light was the conditioned stimulus and the tone was the blocked stimulus, and finally one group received a compound stimulus of tone and light. Once rats were conditioned to the single stimulus, and the compound had been presented, probe trials were used to assess the extent of blocking. During probe trials both stimuli were presented on their own without reward and approaches to the hopper where the reward pellet was delivered were recorded. As expected, responses to the hopper were higher when the conditioned stimulus was presented as compared to the blocked stimulus. Animals in the compound condition showed equal approaches to each stimulus.

After the final testing session animals were perfused and brains were processed for Fos protein. Immunohistochemical analysis revealed significantly greater Fos-protein positive neurons in the sector of the thalamic reticular nucleus that corresponded to the conditioned stimulus. Rats in the compound experimental group showed equal activation across auditory and visual sectors. The significantly greater number of Fos positive neurons in the visual sector of those conditioned to the light compared to those conditioned to the tone show that visual stimulation from the environment is insufficient to recruit the visual thalamic reticular nucleus (McAlonan, Brown, & Bowman, 2000). Such suggests that the thalamic reticular nucleus is involved in attention rather than mere sensory processing.

Petrof and Brown (2010) additionally showed that visual stimulation was insufficient to involve visual thalamic reticular nucleus – increased Fos expression was only seen in those rats performing a visual discrimination task.

While indicating a role for the thalamic reticular nucleus in direction attention towards salient targets, Fos studies are unable to provide insight into the mechanism through which the thalamic reticular nucleus is involved in such tasks. It is hypothesised that one of the actions through which the thalamic reticular nucleus exerts its effects is through lateral inhibition. With extensive inhibitory interconnectivity, activation of thalamic reticular neurons by cortical projections is able to recruit other areas of the inhibitory network (Zhang & Jones, 2004). By suppressing the processing of one stimulus, more resources can be dedicated towards the behaviourally relevant stimulus. The above data suggests that the relevant sensory sector of the thalamic reticular nucleus is only recruited when focus, or attention, is directed towards said sensory dimension. It is unclear whether increased activity reflects increasing attention to the relevant stimulus, inhibition of other sensory sectors, or both.

The current experiment sought to examine the effect of cell body lesions of the visual thalamic reticular nucleus on visual discrimination trials with and without an auditory distraction. Modulation of attention by the thalamic reticular nucleus within a modality has already been established (Weese, Phillips, & Brown, 1999), but it has not yet been shown whether the thalamic reticular nucleus is required to block the processing of irrelevant stimuli in a non-attended modality. It is predicted that lesioning the visual thalamic reticular nucleus will impair the orientation and

maintenance of attention towards the relevant visual stimulus, and therefore an auditory distractor will result in a greater number of errors in lesioned rats.

3.2 Methods

3.2.1 Animals

Eighteen male Lister hooded rats (Charles River, UK) were used within this experiment. Throughout the study period rats were trained on daily 45 minute sessions between 08:00 and 17:00.

3.2.2 Cross Modal Distraction Task

3.2.2.1 Habituation

***Step 1 - Acquisition of nose-poke response*.** A series of five, 1 second flashes were presented in the central hole, followed by a series of five flashes in the hole either immediately to the right or left (location randomly determined). After the flashes, a light above the reward hopper was activated for 3 seconds, during which the reward was presented. The number of pokes in the central and flanker holes was recorded. The session was terminated by the experimenter once the rat was reliably poking in all of the 3 holes. All animals completed this stage within 3 days.

***Step 2 - Shaping procedure*.** In this stage the rat is required to make a 600ms central nose poke followed by a poke into one of two flanker holes, according to the location of a temporally unpredictable light in one of the flanker holes. In the first five trials of every session the central hole stayed on until the rat made a central nose-poke response. Once a central nose-poke response had been made, one of the flanker holes was lit for 150ms, and remained on until the rat withdrew its nose and made a nose-poke response. Each correct response was rewarded. Incorrect responses were not punished at this stage. Each session terminated after 80 correct

responses. When the rat completed the session in under an hour they were progressed to the next stage. All animals completed this stage within 15 days.

Step 3 - Two choice visual discrimination task. Rats were required to make a central nose poke for 600ms. The end of this foreperiod was signalled by the appearance of a light coming on above or below the rat. The response required based upon location was counterbalanced, so that for some rats the light above required a nose poke immediately right to the central hole, and a below required a nose poke to the left. For the other group of rats a light above required a left response, and a light below a right response. A late response was recorded if the rat failed to respond 3 seconds after the onset of the light. A new trial was initiated once reward had been collected. After incorrect or no responses, the timeout period was ended by the central nose hole being illuminated, signalling the initiation of a new trial. Incorrect trials were repeated until a correct response was made. Training in this final stage was terminated when the rat reached 80% criterion, which equated to 128 correct responses out of the 160 trials per session. Aii animals completed this stage within 16 days.

3.2.2.2 Testing

The testing phase of the experiment comprised two sessions. One session was the same as the final testing session where all trials were visual discrimination trials without distractors, while the other session contained (i) non-distractor, (ii) white noise distractor, and (iii) tone distractor trials. These sessions were alternated during baseline data collection, and again post-surgery.

Non distractor trials: The rat was required to make a central nose poke for a duration of 1.25s. The end of this foreperiod was signalled by the appearance of a light coming on above or below the rat. During training rats learned the response required as a function of location. When the light appeared the rat had to make the desired response, a correct response resulting in a reward and an incorrect a timeout.

Distractor trials : The response required by the rat in distractor trials was identical to that in non-distractor trials. The only difference was that in the distractor trials either white noise or a short burst of 3 tones were presented with the visual stimulus. The rat simply had to ignore the auditory stimulus and make the response dictated by the visual stimulus (See figure 3.1)

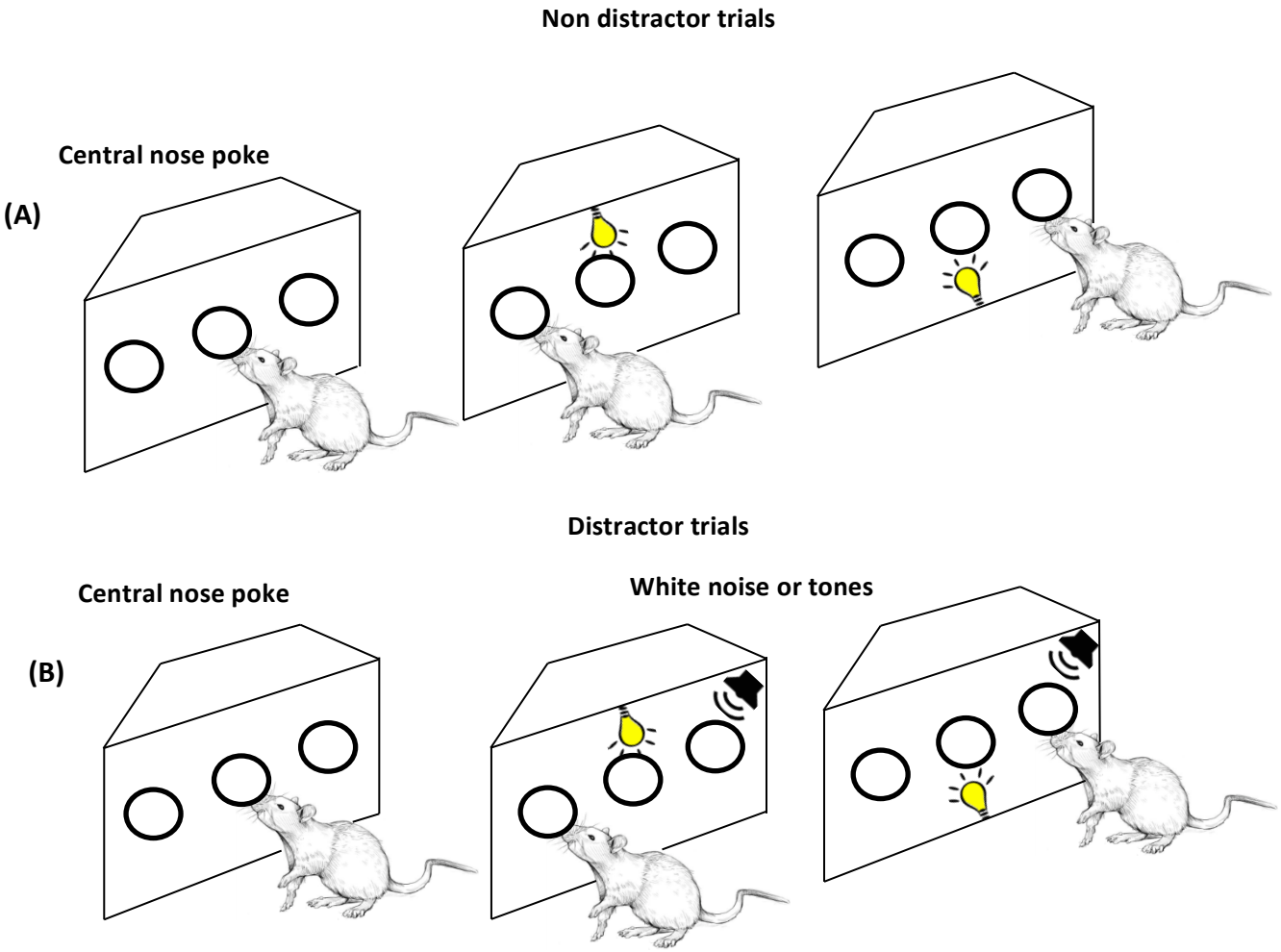


Figure 3.1. Schematic representation of task requirements in the cross modal distraction task. As this cross-modal task is a relatively new task, the decision was made to include two different distractors. It was not known which of the distractors would prove the most efficacious. In order to eliminate the need to replicate the study with a new distractor should one prove ineffective, it was deemed appropriate to include two distractors. With 160 trials per session there was adequate opportunity to collect sufficient data regarding reaction time for each of the three trial types.

3.3 Surgery

Twelve rats received bilateral visual thalamic reticular nucleus lesions with 0.15µl 0.09M ibotenic acid at coordinates AP -3.3mm, ML ± 3.5mm, DV -5.8mm (from skull surface) using a 25 gauge Hamilton syringe. A further six rats received bilateral sham lesions of 0.15µl 0.1M PB into visual thalamic reticular nucleus at

coordinates AP -3.3mm, ML ± 3.5mm, DV -5.8mm (from skull surface). Both toxin and PB were injected over 3 minutes and the needle left in situ for 3 minutes.

3.4 Immunohistochemistry

After perfusion, brain removal and preparation tissue sections were stained for neuronal nuclei (see general methods for details).

3.5 Data Collection and Analysis

Data pertaining to correct and incorrect responses were collected, allowing for the calculation of the percentage correct. Repeated measures ANOVAS were used to analyse percentage correct performance with trial type (non-distractor, white noise, and tone) and surgery (pre and post) as the within subjects factor, and lesion group as the between subjects factor.

3.6 Results

3.6.1 Immunohistochemistry

Staining for neuronal nuclei was conducted. All lesion animals sustained bilateral visual thalamic reticular nucleus lesions. No damage to the visual thalamic reticular nucleus in the sham animals was seen (see figure 3.2).

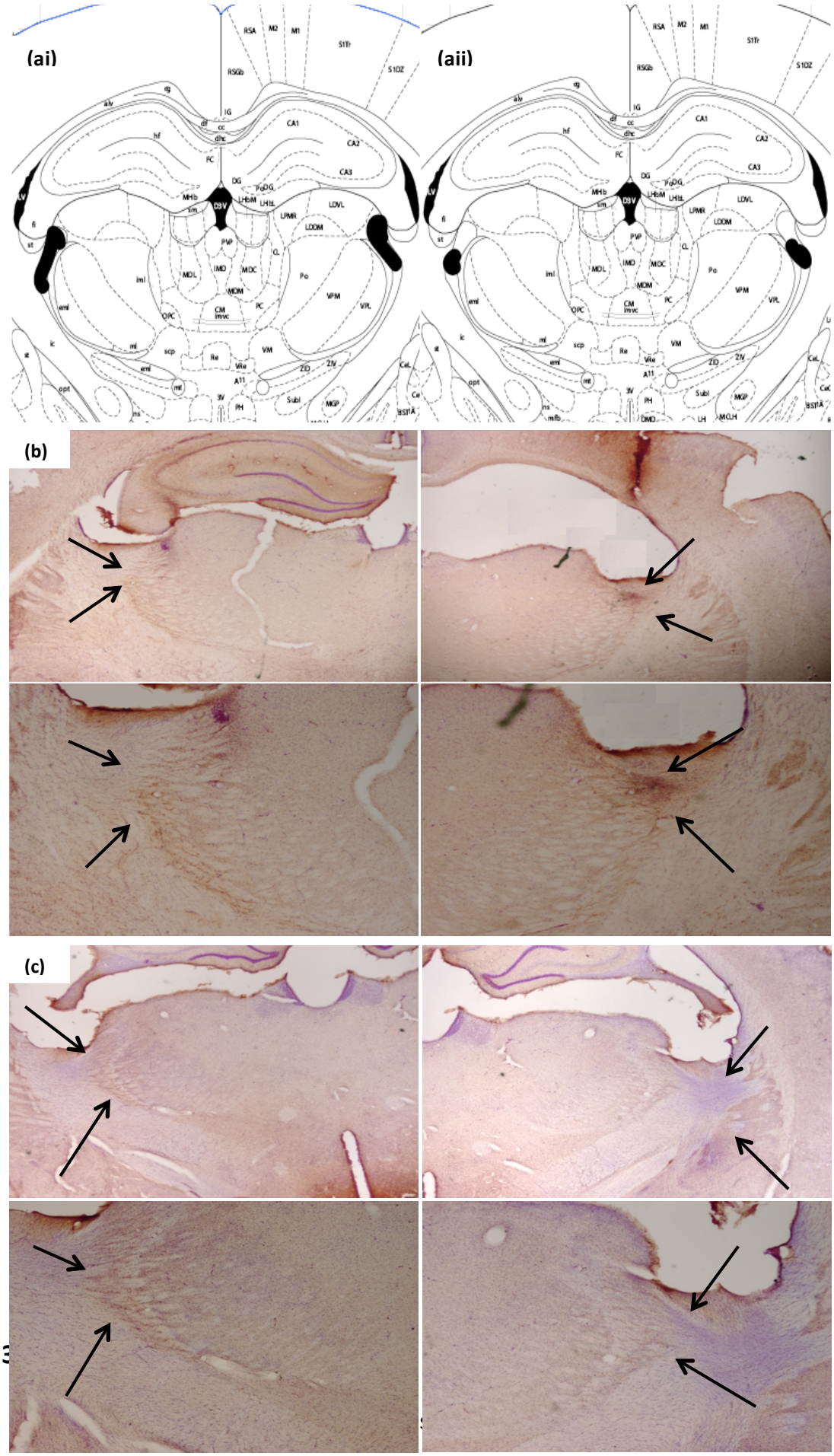


Figure 3.2.Schematics showing the largest (ai) and smallest (aii) visual thalamic reticular nucleus lesions. Example of bilateral visual thalamic reticular nucleus lesion (b) and sham bilateral visual thalamic reticular nucleus lesion (c)

noise and tone auditory distractor trials (significant main effect of trial type [F(3,48)=43.764, p<.01]). White noise trials were associated with the most errors. This confirms that the white noise and tones were sufficiently distracting.

Following surgery the same behaviour pattern was shown, with distractor trials having the lowest number of correct responses. Performance between lesion and sham animals did not significantly differ (non-significant trial type*surgery interaction [F(3,48)= 2.314, n.s.]), demonstrating that the visual thalamic reticular nucleus lesions did not increase the distractibility of the irrelevant auditory stimuli (See figure 3.3).

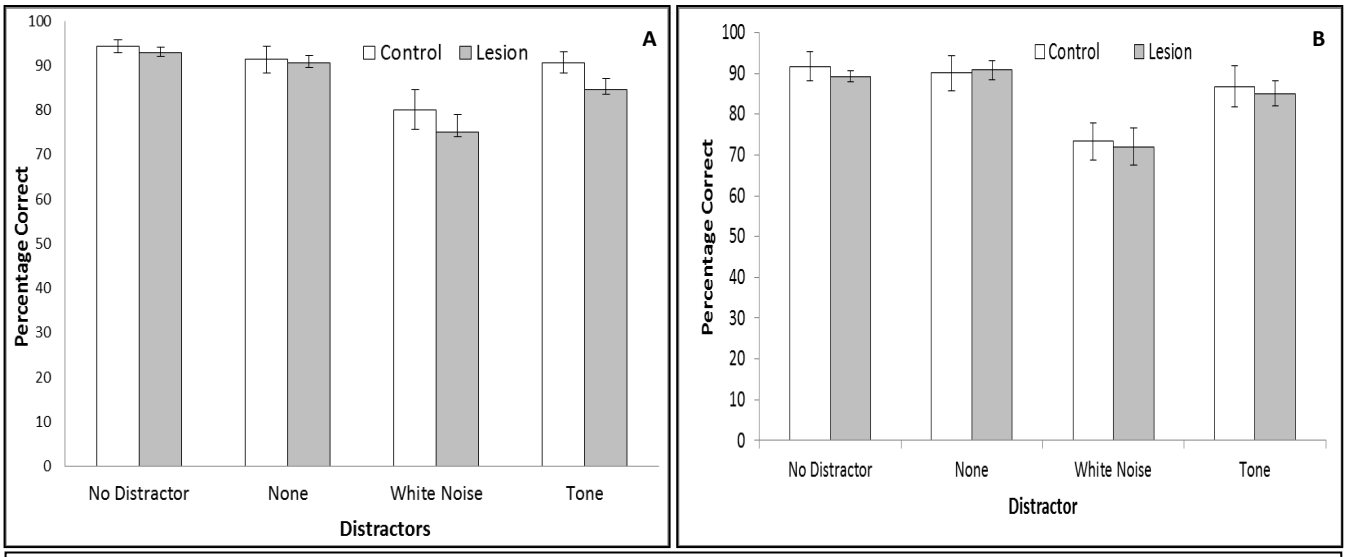


Figure 3.3. (A) Pre surgery performance (B) Post surgery performance in the cross modal distraction task.No distractor trials were sessions with only no distractor trials whereas none are tials with no distractors in a session with other distractor trials.

3.7 Discussion

The current experiment aimed to examine whether lesions of the visual thalamic reticular nucleus would increase the distractibility of an auditory stimulus presented simultaneously with a visual stimulus. It was predicted that the lesions would compromise attention directed towards the visual stimulus, which would in turn make the auditory stimuli more distracting. Although the auditory stimuli presented were found to be distracting as evidenced by a reduction in the number of correct responses in their presence, visual thalamic reticular nucleus lesions did not increase their distractibility as compared to controls.

The hypothesis was that lesions would increase distractibility, which was based upon assumptions about the possible mechanism of lateral inhibition. Activation of the sensory sector of the thalamic reticular nucleus corresponding to the attended stimulus observed in the McAlonan, Brown, & Bowman (2000) blocking experiment could be interpreted as serving, by lateral inhibition, to down-regulate other potential channels of information. More specifically, if activation of the visual sector of the thalamic reticular nucleus actively inhibit activity in the auditory sector, this could enhance transmission of sensory information pertaining to the salient (light) stimulus and decrease transmission of information from not-relevant modalities. If this were the case, the prediction was that lesions would have resulted in increased distractibility, and therefore a significant reduction in percentage correct during the white noise and tone trials. However, we did not see evidence of any reduction in percentage correct.

After a series of sessions, the auditory distractors are less distracting: they do not require any response other than to be ignored, which may become easier as they are more familiar. Nevertheless, there remained a significant effect of both white-noise and the tone distractor and this effect did not change following the lesion. The fact that the tone was slightly less disruptive than the white noise gives us confidence that the rat is not performing 'at floor' – that is to say, the rats were not maximally distracted by both such that a subtle lesion effect could not have been detected.

A remaining possibility is the lesion of the visual area of the thalamic reticular nucleus was insufficiently complete so that remaining functionality was sufficient to mask a lesion effect. However, this explanation is not very strong. It is almost impossible to produce a lesion which encapsulates the entire region of interest without producing some damage to surrounding areas. Therefore, all researchers must accept that some neurons will be spared whenever lesions are produced. It would be naïve to suggest that an incomplete lesion can always explain a lack of significant impact on behaviour, and therefore this is not a satisfactory explanation. In this task the rat merely had to try not to be distracted. The two auditory distractors used (tones or white noise) were of no significance to the rat as before the task they had not previously been exposed to them. Their lack of salience could possibly explain the pattern of results found. It is possible that the lack of significance attributed to the sounds meant that bottom up processes were sufficient to deal with their presence. Clearly the tones and white noise were distracting, but following lesions to visual thalamic reticular nucleus their presence did not become more detrimental to performance. Evidence suggests that the

thalamic reticular nucleus does not merely respond to the presence of environmental stimuli, but rather is involved in attentional filtering and the allocation of cognitive resources to pertinent stimuli (Petrof & Brown, 2010). This allocation of attentional resources is a top-down process, serving to ensure optimal performance.

Disconnect between the distractors and task requirements could possibly explain a lack of top down engagement. Consider a scenario where you are having a conversation with another person. If you hear somebody sneeze in the background you are unlikely to become distracted and miss elements of the conversation because it is not a very salient sound. However, if you hear/feel your phone vibrating you may suddenly find yourself distracted even if you do not intend answering the phone. Your phone vibrating is far more salient to you than a stranger's sneeze and therefore requires much more effort to actively ignore. Perhaps if the distractor had more significance to the rat -being used beforehand, or signifying another action required elsewhere – then top-down control would have been required and the lesion would have had an effect.

Finding a way to make the auditory distractors have more significance, while maintaining the essence of the task would need to be carefully considered. However, a task already designed which maintains some aspect of the issue discussed is the attentional set shifting task. Although a very different task, especially from a methodological perspective, the attentional set shifting task has reversal and shift stages in which the rat has to suppress responding to a previously rewarded dimension. Although the tasks themselves are not comparable, its clear top-down recruitment evidenced by studies showing strong prefrontal involvement (Birrell &

Brown, 2000; McAlonan & Brown, 2003) make it an interesting task to consider when studying the thalamic reticular nucleus. During the reversal and extradimensional shift stages stimuli that were previously rewarded become irrelevant, meaning the rat must actively ignore them and make responses based on other dimensions. Chapter 5 details the effects of rostral thalamic reticular nucleus and mediodorsal thalamus lesions on performance in the attentional set shifting task.

Excitotoxic lesions of the thalamic reticular nucleus do not impair performance on the attentional set shifting task

Abstract

Strong reciprocal connections between the thalamic reticular nucleus and both cortex and thalamus make it an ideal candidate for involvement in attentional processes. Previous studies have shown that the thalamic reticular nucleus is involved in attentional orienting of visuospatial information. However to date it has not been established whether the thalamic reticular nucleus is involved in other tasks of attention. Lesions of prefrontal cortices have resulted in deficits in attentional set shifting performance and it is possible, considering the connectivity between the thalamic reticular nucleus and these structures, that mechanisms required for optimal performance involve the thalamic reticular nucleus.

The aim of this study was to determine whether excitotoxic lesions of the thalamic reticular nucleus lesions would impair performance on the attentional set shifting task, performance of which is known to be prefrontal dependent. Rats received bilateral thalamic reticular nucleus lesions using ibotenic acid. Performance of the lesion animals did not differ to that of the control animals, suggesting that lesions of the thalamic reticular nucleus do not impair performance on the attentional set shifting task.

4.1 Introduction

Performance in the attentional set shifting task, as detailed by Birrell & Brown (2000) has been reliably shown to be prefrontal dependent. Although the thalamic reticular nucleus has been implicated in orienting of visuospatial attention, there have been no definitive studies of its role in the formation, or shifting, of attentional set. By virtue of its connectivity with both thalamus and cortex, the thalamic reticular nucleus is ideally placed to be involved in optimal performance in this task of attention.

There is some evidence to suggest that the thalamic reticular nucleus may be involved in attentional set shifting. Although the task used was not that detailed by Birrell and Brown (2000), Petrof & Brown (2010) showed increased thalamic reticular nucleus activity in the sensory sector of rats associated with the dimension of their assigned discrimination. During the task, rats were made to discriminate between bowls based on either their visual (colour of bowl) or tactile (texture of bowl) characteristics in order to obtain a food reward. Rats were perfused following their final testing session, in which they were tasked with completing one of the two previously mentioned discriminations. There was significantly greater activation, meausured by c-fos positive neurons, in visual thalamic reticular nucleus as compared to somatosensory thalamic reticular nucleus in those rats who performed the visual discrimination prior to perfusion. Interestingly, there was no corresponding increased activation in the sensory thalamic reticular nucleus in those rats that performed the tactile discrimination prior to perfusion. Although the results from this study suggest that the thalamic reticular nucleus may be involved in

individual discrimination stages similar to those used for attentional set shifting, only one pair of tactile and visual stimuli were used. Therefore, while reversals could be used there was no opportunity to form attentional set as multiple sets of each variation would have been needed.

Modelled on the ID/ED CANTAB task (Cambridge Cognition), the attentional set shifting paradigm can be used as a test of cognitive deficits in a wide variety of rodent models of neurological disorders such as Huntington's disease (Brooks, Betteridge, Trueman, Jones, & Dunnett, 2006), and Schizophrenia (Rodefer, Murphy, & Baxter, 2005).

PCP administration is often used as a model of the cognitive deficits observed in Schizophrenia. Egerton, Reid, McKerchar, Morris, and Pratt (2005) found that reduced parvalbumin expression in the thalamic reticular nucleus was significantly correlated with deficits in extradimensional shifts. More specifically, animals required more trials to reach criterion at the extradiemsional stage – the authors proposing that perturbations in the ability of the thalamic reticular nucleus of PCP treated to control the relay of sensory information as an explanation of the results. Performance in the attentional set shifting task has been shown to be prefrontal dependent – lesions of orbital prefrontal cortex impairing performance at reversal stages (McAlonan & Brown, 2003), while lesions of medial prefrontal cortex impair shifting of attentional set (Birrell & Brown, 2000). Connections between the thalamic reticular nucleus and prefrontal areas may be involved in performance on this task. By damaging prefrontal areas, and therefore severing/impairing connectivity, the thalamic reticular nucleus may not be able to adequately control the transmission of relevant sensory information.

In order to successfully form attentional set, attention must be directed towards a single stimulus dimension (odour for example), while simultaneously inhibiting the processing of a second, irrelevant dimension (Birrell & Brown, 2000). As both the relevant and irrelevant stimuli are presented in a multidimensional format, performance requires filtering of irrelevant information to optimise the processing of relevant information (Tait, Chase, & Brown, 2014). The thalamic reticular nucleus is widely proposed to be the means through which incoming sensory information from the thalamus is filtered before being transmitted to cortex (McAlonan & Brown, 2002). It is therefore plausible to propose that the thalamic reticular nucleus is involved in the formation and shifting of attentional set.

In light of the above, it is anticipated that lesions of thalamic reticular nucleus will result in impairments in performance on the attentional set shifting task. Given the centrality of the thalamic prefrontal circuit to cognition, there are two possible outcomes – lesions could result in impairment the shifting of attentional set, or reversal learning.

4.2 Methods

4.2.1 Animals

Twelve male Lister hooded rats (Charles River, UK) were used within this experiment. Throughout the study period rats were trained on daily 60 minute sessions between 08:00 and 17:00.

4.2.2 Apparatus

The test apparatus used in the attentional set-shifting paradigm was constructed from a large homecage (see general methods for images). An acrylic panel was placed one third down the cage, which is itself divided into two sections of the same size. Digging bowls were placed in the two smaller compartments, with the larger section used as a free area for the rat to sit and consume its reward between trials. One small and one large removable acrylic panel were used to separate the rat from either or both sections, respectively. To grant access to the rat the panel was lifted from the slot.

The digging bowls were ceramic bowls with a depth of 4cm and internal diameter of 7cm. The bowls were filled with various digging media which could be scented with various spices and herbs (See table 4.1). In each trial half a honey loop (Kellogg Company, UK) was buried 2cms below the surface of the medium in the centre of the bowl.

For the visual discrimination task, digging bowls with horizontal and vertical black and white stripes were used. Consistent with the attentional set shifting apparatus, they were ceramic bowls with a depth of 4cm and an internal diameter of

7cm. The bowls were filled with sawdust. In each trial half a honey loop (Kellogg Company, UK) was buried 2cm below the surface of the sawdust in a central location.

Pair	Odour	Medium
1	O1 Cinnamon, O2 Ginger	M1 Coarse tea, M2 Fine tea
2	O3 Sage, O4 Paprika	M3 Sand, M4 Grit
3	O5 Turmeric, O6 Clove	M5 Coarse sawdust, M6 Fine sawdust

Table 4.1. A list of all odours and digging media used in the 7-stage attentional set-shifting task.

4.2.3 Attentional Set-Shifting Task

4.2.3.1 Habituation and Training

The day before training two ceramic bowls containing sawdust and six honey loops at the bottom, were placed in the home cage. All of the honey loops were consumed by the following morning.

During training rats were put in the waiting area section of the set shifting box. A ceramic bowl containing sawdust and half a honey loop placed on top was left in each of the two small sections. The large panel was removed, thus allowing the rat to explore both bowls and find and eat the reward. In the proceeding five trials the reward was placed progressively further from the surface of the sawdust until the rat was reliably digging to find the reward. During each trial, if the rat didn't find the reward within ten minutes the panel was placed down and a new trial was initiated with the timer set back to zero.

For the second stage of training, the rat performed a simple discrimination between two bowls filled with normal sawdust. One was scented with mint and the other oregano. The rats had to dig and learn which one was baited. The bowls were placed in the two small compartments, with only one scent paired with the reward. The side of placement was randomly determined for each trial, but there could be no more than three instances where the correct bowl was on the same side. Rats were given up to ten minutes to uncover the reward, after which the trial was ended and the next trial initiated. If the rat first dug in the correct bowl, the latency from panel withdrawal and digging was written down and the trial was recorded as correct. If the rat first dug in the incorrect bowl, the latency from panel withdrawal and digging was written down and the trial was recorded as incorrect. If an incorrect response occurred within the first four trials of the stage, then the panel was placed over this compartment, and the rat was allowed to recover the reward from the correct bowl. After the first four trials however, access to both compartments were blocked off from the rat and a new trial was started. The order at which the rat approached the bowls was also noted down. The criterion for learning was six consecutive correct trials, which included the first four self-correct trials. Training took place no more than 3 days before testing.

4.2.3.2 Testing – 7-Stage task

The 7-stage attentional set shifting task was first described by Birrell & Brown (2000), comprising seven discrimination stages. The first stage was the simple discrimination (SD), in which rats were required to discriminate between two exemplars from a single dimension (odour or medium). A compound discrimination

(CD) followed, in which the rewarded exemplar was the same from the previous stage, but a second irrelevant perceptual dimension was added. A reversals stage (Rev1) followed in which the stimuli from the compound discrimination remained, but the correct and incorrect responses were reversed (if cinnamon was correct and ginger incorrect in the CD, then ginger become the correct response). During reversal stages animals are required to maintain attentional set pertaining to a specific dimension, as well as learn, and simultaneously unlearn, stimulus-reward associations. Subsequently there was an intradimensional shift, where all exemplars were replaced, but the attended perceptual dimension was the same. This was followed by a second reversal (Rev2) in which the correct and incorrect responses were again reversed. A final set of new exemplars was then introduced during which the previously incorrect dimension became rewarded – this stage was known as the extradimensional shift. The difference in trials to criterion between the ID and ED stages is referred to as the shift cost, and reflects the ability to totally shift set. It is expected that sifting set from one perceptual dimension to another is more difficult than shifting within a dimension. The final stage comprised a third reversal (Rev3). As with training, rats were required to achieve six correct consecutive trials to progress to the next stage, with the first four trials being nominated as "self-correct" trials (See table 4.2 for an exemplar procedure).

Stage	Discrminanda	Paired with
Simple discrimination	M1 (Coarse tea), not M2 (Fine tea)	No pairing
Compound discrimination	M1 (Coarse tea), not M2 (Fine tea)	O1 (Cinnamon), or O2 (Ginger)
Reversal 1	M2 (Fine tea), not M1 (Coarse tea)	O1 (Cinnamon), or O2 (Ginger)
Intradimensional discrimination	M3 (Sand), not M4 (Grit)	O3 (Sage), or O4 (Paprika)
Reversal 2	M4 (Grit), not M3 (Sand)	O3 (Sage), or O4 (Paprika)
Extradimensional discrimination	O5 (Clove), not O6 (Turmeric)	M5 (Coarse sawdust), or O6 (Fine sawdust)
Reversal 3	O6 (Turmeric), not O5 (Clove)	M5 (Coarse sawdust), or O6 (Fine sawdust)

Table 4.2 . An example of the stages and stimuli used in the 7-stage attentional set shifting task. The stages were always run in the same order, with the correct stimuli counterbalanced across rats.

4.2.4 Visual Discrimination Task

It was foreseen that the lesions produced may encroach on the visual sector of the thalamic reticular nucleus. In order to determine whether this would impair performance, a simple high contrast visual discrimination was added to the protocol. After completion of the 7-stage attentional set shifting task, the rats performed a simple discrimination stage followed by a reversal stage.

The protocol mirrored that used in the attentional set shifting task. Animals were placed in the waiting area of the testing box, with a acrylic barrier stopping them accessing either of the bowls. The bowls differed only in the orientation of the black and white stripes. When the barrier was removed, the rat had access to both bowls. Only one of the bowls was baited (baiting of horizontal or vertical as simple

discrimination was counterbalanced), and the rats had to dig to learn which had the food reward. The location of the bowl containing the reward was randomly determined for each trial, but there could be no more than three instances where the bowl was at the same side. Rats were allocated ten minutes per trial to uncover the reward. If they failed to make a response in this time the trial was ended and a new trial initiated. The latency to dig in was recorded, as well as whether this was the first or second bowl they approached. If the rat first dug in the baited bowl, the time from panel withdrawal was recorded, and the trial was marked correct. If the rat first dug in the unbaited bowl, the time from panel withdrawal was recorded, and the trial was marked incorrect. If an incorrect response occurred within the first four trials of the stage, then the panel was placed over this compartment, and the rat was allowed to recover the reward from the correct bowl. After the first four trials however, access to both compartments were blocked off from the rat and a new trial was started. The order at which the rat approached the bowls was also noted down. The criterion for learning was six consecutive correct trials, which included the first four self-correct trials.

Upon completion of the first simple discrimination stage, a reversal stage followed. This stage only differed in that the previous unrewarded bowl became the baited bowl.

4.2.5 Surgery

Eight rats received bilateral thalamic reticular nucleus lesions with 0.2µl of 0.06M ibotenic acid at coordinates AP -3.4, ML ± 3.9 DV -4.9 (from dura) via pulled glass pipette as a bolus infusion. The pipette was left in situ for 3 minutes. Four

control animals received an injection with 0.2µl of 0.1M PB into thalamic reticular thalamus at coordinates AP -3.4, ML ± 3.9 DV -4.9 (from dura) via pulled glass pipette as a bolus infusion. Intraperitoneal injection of 0.25ml diazepam was administered after the animal was anaesthetised.

4.2.5.1 Immunohistochemistry

After perfusion, brain removal and preparation, tissue sections were stained for neuronal nuclei (see general methods for details).

4.3 Data Collection and Analysis

Data pertaining to trials to criterion, errors to criterion, dig latency and the number of non-dig trials was recorded. However, only trials to criterion and errors to criterion were analysed. It was not anticipated that either lesion would impair performance at the SD, CD and ID stages, a priori hypotheses were limited to impacts on performance at reversals, and an increased shift cost. Repeated measures ANOVAS were used to analyse performance with stage as the within subjects factor, and lesion group as the between subjects factor. Due to a priori predictions, separate RM ANOVAs compared both trials and errors to criterion across all 7 stages of the task, with additional ANOVAS being restricted to comparing performance across reversal stages, or between ID and ED stages.

For the visual discrimination task repeated measures ANOVAS were used to analyse performance with stage as the within subjects factor, and lesion group as the between subjects factor.

4.4 Results

4.4.1 Immunohistochemistry

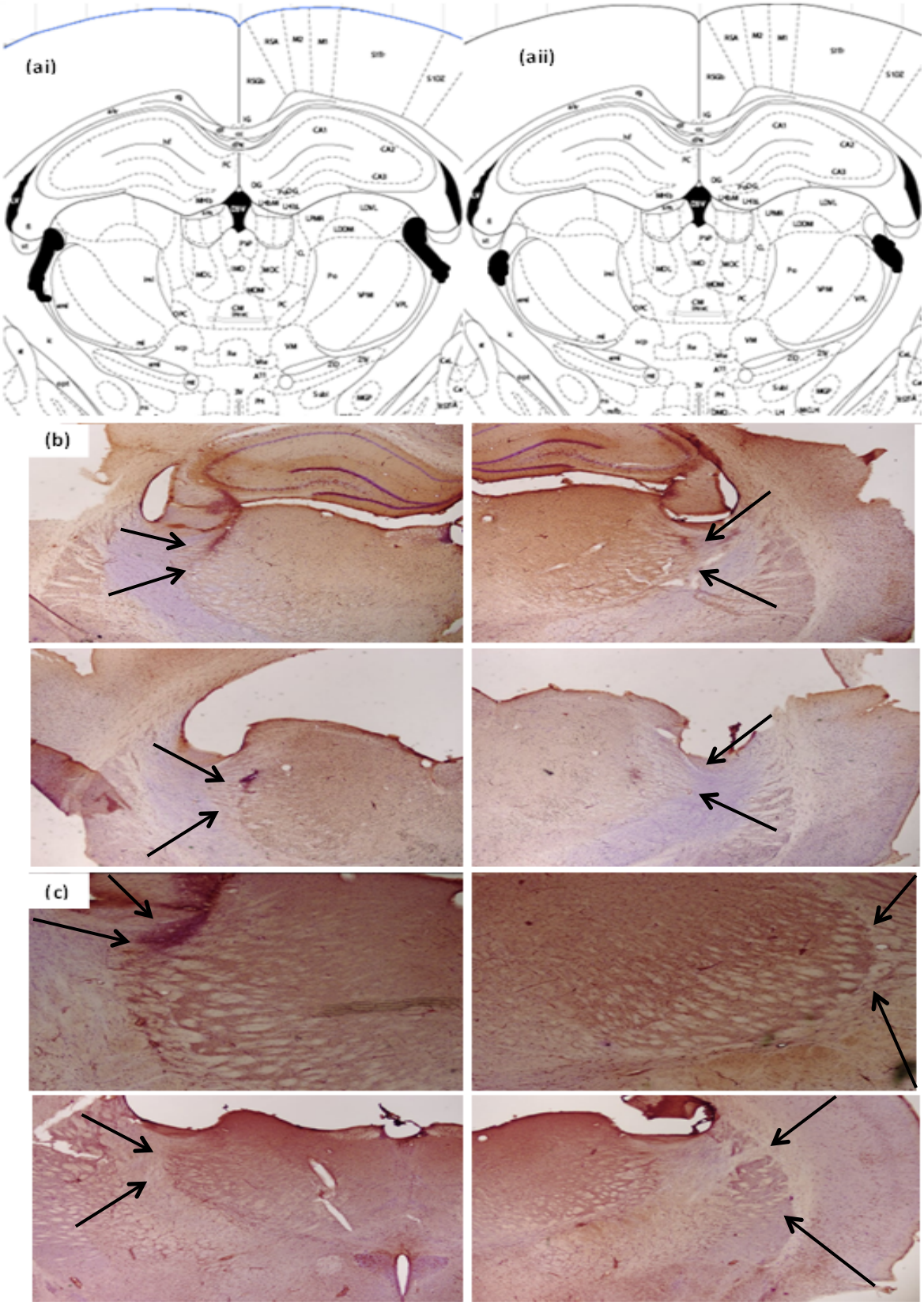


Figure 4.1. Schematics showing the largest (ai) and smallest (aii) thalamic reticular nucleus lesions. Example of bilateral thalamic reticular nucleus lesion (b) and sham bilateral thalamic reticular nucleus lesion (c)

4.4.2 Attentional Set Shifting Task

The rats required a different number of trials to reach criterion according to the particular discrimination, as shown in Figure 4.2. In particular, reversals took more trials than acquisition of new discriminations. This was confirmed by a main effect of Stage ($F_{(6,60)} = 3.95$, $p < 0.05$, $\eta_p^2 = 0.28$, Observed Power = 0.96) and planned contrasts between different stages. All three reversals had significantly more trials that any of the acquisition stages (SD, CD, ID and ED), however the acquisition stages did not differ from each other. There was no difference between the groups at any stage. This was confirmed by the lack of an, adequately-powered, interaction of Stage x Group ($F_{(6,60)} = 1.3$, n.s., $\eta_p^2 = 0.12$, Observed Power = 0.48)

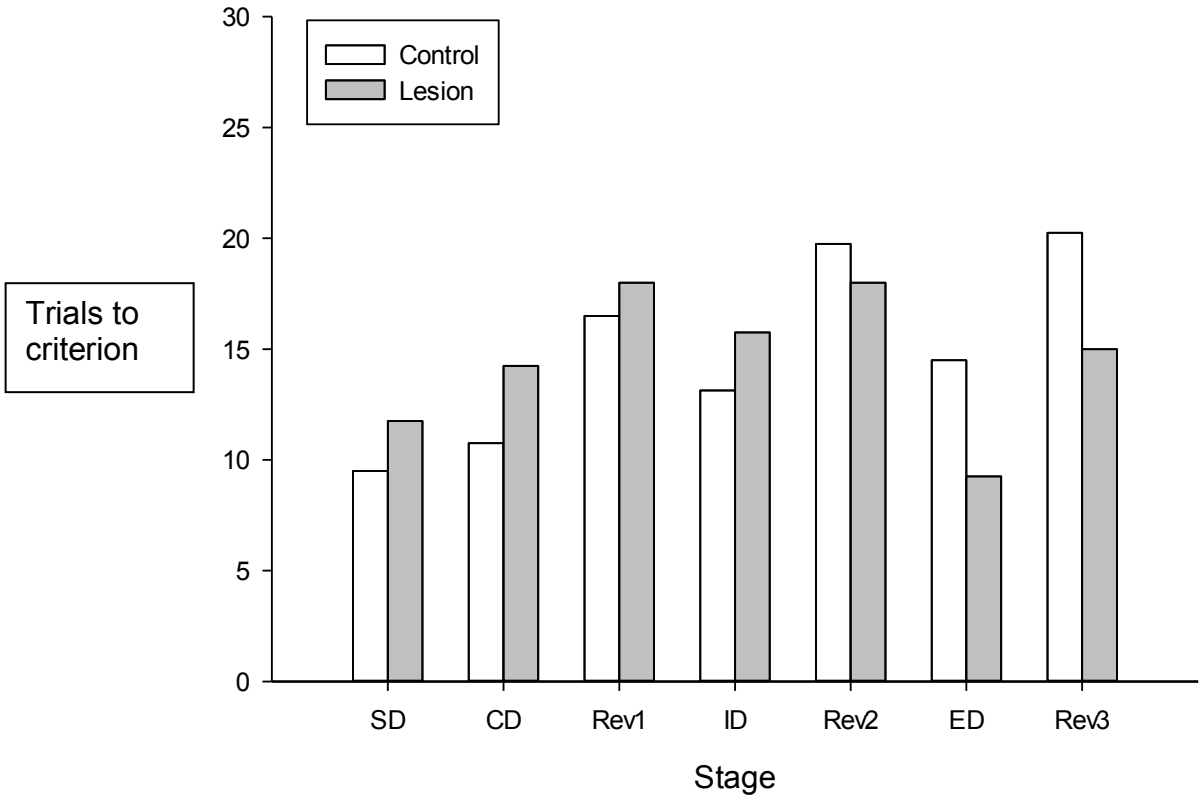


Figure 4.2 . Mean (+S.E.M.) trials to criterion are plotted for each stage of the 7 stage task, with the stages shown in the order in which they were presented (left to right). White bars are control data and filled bars are the lesion group. There was no significant difference between the groups at any stage

As expected, the same pattern of results was found for Errors to Criterion (data not shown), with more errors in the reversal stages than the acquisition stages, which did not differ from each other. There were also no main effects of, or interactions with, Group (Fs < 1.0).

4.4.3 Visual Discrimination Task

The 7 stage attentional set shifting task does not rely on vision, however it is possible that the lesion might have encroached into the visual area of TRN and a visual deficit could not be ruled out. Therefore, the rats were also tested on a two-choice visual discrimination between horizontally and vertically striped (i.e., a high contrast discrimination) digging bowls.

The rats were much less good, and performance was more variable, at this simple visual discrimination and reversal, requiring more than twice as many trials to reach criterion (mean 26.3, S.D. = 12.9) compared to odour or digging media discriminations, which were typically completed within 12 trials. Nevertheless, there was no evidence of an impairment in the lesion group and for both the main effect of Group and interaction of Stage x Group the F-ratio was < 1.0. The reversal stage was completed in slightly fewer trials than the acquisition, but this was not a statistically significant difference ($F_{(1,10)}$ = 2.0, n.s., η_p^2 = 0.17, Observed Power = 0.25) therefore no inferences can be drawn from this.

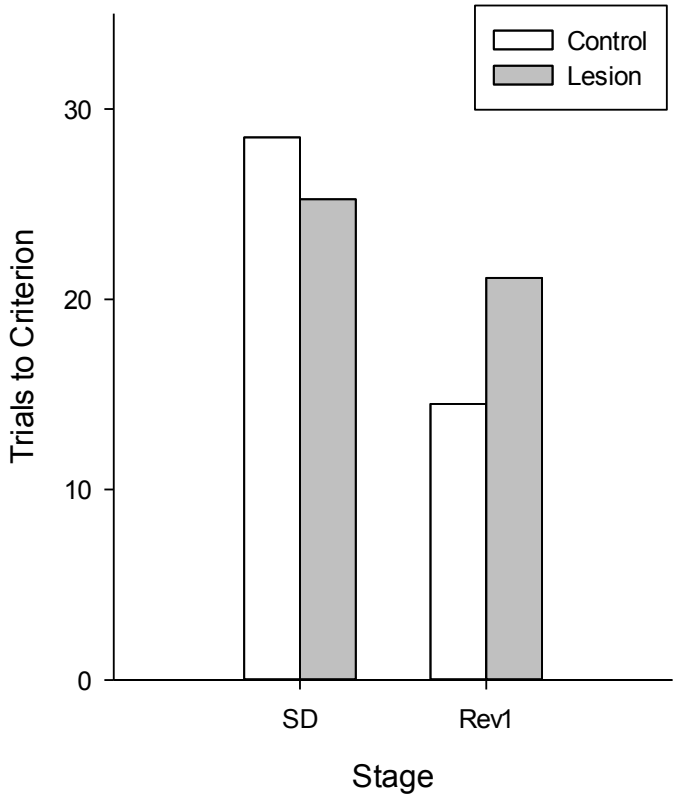


Figure 4.3 . Mean (+S.E.M.) trials to criterion are plotted for each stage. White bars are control data and filled bars are the lesion group. There was no significant difference between the groups at any stage

4.5 Discussion

The aim of this chapter was to examine the effects of bilateral ibotenic acid lesions of thalamic reticular nucleus on performance in the attentional set shifting task. The sensitivity of the task to highlight cognitive impairments following prefrontal lesions, and connectivity between cortex and the thalamic reticular nucleus, it was predicted that similar deficits would be seen following thalamic reticular nucleus. However, it was found that the performance of lesion animals did not significantly differ from that of the control animals.

Although one might be tempted to conclude that lesions of the thalamic reticular nucleus do not impair attentional set, the overall performance of the animals complicates drawing definitive conclusions. The "traditional" pattern of performance was not demonstrated, namely no significant difference in the number of trials required to reach criterion between the intradimensional (ID) and extradimensional (ED) stages. In the absence of an ID/ED difference in the control group, it is not possible to draw inferences about the existence of an attentional set or indeed, the effect of this lesion on attentional set. That is to say, even if the lesion may have impaired set-shifting, the lack of evidence of set means that no conclusions can be drawn.

Additionally, there was no evidence of any impairment more generally in the test. Although a simple high-contrast visual discrimination appeared to be more difficult for the rats than odour or medium discriminations, this was true regardless of the lesion. The particular difficulty could have, in part, been due to prior experience of performing discriminations between bowls based on their odours or

digging media. Nevertheless, Petrof and Brown (2010) also reported trials to criterion for a visual discrimination of around 20, so these results are not considered abnormal. Lister-hooded rats, although not albino, do not have good vision and therefore testing them in normal levels of lighting in the testing room would make even a high contrast discrimination challenging.

Moving forward, research still needs to focus on the role of the thalamic reticular nucleus in the formation and shifting of attentional set. Commenting on the full task is complicated by the lack of attentional set formation, but there are other avenues to pursue. In addition to its sensory sectors, the thalamic reticular nucleus has a non-sensory rostral sector. Often referred to as the cognitive sector, the rostral thalamic reticular nucleus has strong reciprocal connections with the mediodorsal thalamus (Cornwall, Cooper, & Phillipson, 1990). Additionally, the mediodorsal thalamus has strong connections with the medial prefrontal cortex (Groenewegen, 1988), which has been shown to be involved in the shifting of attentional set (Birrell & Brown, 2000). It is possible that rather than being involved in the sensory aspects of the task, the thalamic reticular nucleus is involved in the overarching formation or shifting of attentional set.

Successful formation of attentional set requires the deciphering of relevant from irrelevant sensory input from complex multidimensional stimuli. This information must then be abstracted and processed in reference to behaviourally relevant (and therefore rewarded) features. If the thalamic reticular nucleus is involved in this higher level of processing, then the rostral sector of the thalamic reticular nucleus is the likely source. Connectivity with other “cognitive” thalamic areas, as well as prefrontal areas makes it a strong candidate. This hypothesis is

examined in Chapter 5 by comparing the effects of excitotoxic lesions of the rostral thalamic reticular nucleus and mediodorsal thalamus on the formation, maintenance, and shifting of attentional set in the 7 stage attentional set-shifting task.

Lesions of the rostral thalamic reticular nucleus or mediodorsal thalamus do not impair performance on the attentional set shifting task

Abstract

The thalamic reticular nucleus has previously been implicated in orienting of visuospatial attention. However, it has yet to be established whether the thalamic reticular nucleus is also involved in shifts of attentional set. The rostral, or cognitive, thalamic reticular nucleus has reciprocal connections with the mediodorsal thalamus, which in turn is connected to medial and orbital prefrontal cortex. Both prefrontal areas have previously been associated with performance in the attentional set shifting task – lesions of medial prefrontal cortex impairing shifting attentional set, and orbital prefrontal cortex lesions impairing reversal learning while leaving set shifting ability intact.

Studies of working memory have shown that lesions of the mediodorsal thalamus impair performance in tasks that are prefrontal dependent. The aim of this study was to determine whether mediodorsal thalamus and rostral thalamic reticular nucleus lesions would impair performance on the attentional set shifting task, performance of which is known to be prefrontal dependent.

Rats received either bilateral mediodorsal thalamus or rostral thalamic reticular nucleus lesions using ibotenic acid. Neither lesion impaired the ability of the animals to form, or shift, attentional set. These results suggest that despite

connectivity with cortical areas known to be involved in attentional set shifting performance, the thalamic reticular nucleus nor mediodorsal thalamus are directly involved in acquiring task contingencies or shifting attention.

5.1 Introduction

While the thalamic reticular nucleus has been implicated in the orientation of attention in tasks that measure visuospatial attention, there is little evidence of its role in performance in the attentional set shifting task detailed by Birrell & Brown (2000).

Petrof & Brown (2010) examined c-fos activation in the thalamic reticular nucleus in a discrimination task using stimuli similar to those used in the attentional set shifting task. Animals had to discriminate between bowls based on their tactile (bowl texture) or visual (bowl colour) characteristics. Greater c-fos activation in the visual sector of the thalamic reticular nucleus was found in those animals that performed a visual discrimination one hour prior to perfusion. There was no corresponding activation of somatosensory thalamic reticular nucleus in those animals that performed the tactile discrimination. This task relied purely on discrimination learning and did not require the formation of attentional set as there was only ever one visual and one tactile pair of stimuli used. While there was the possibility for a reversal stage, there was no means to form attentional set as multiple stimuli sets would have been required.

The very nature of the attentional set shifting paradigm means that it can be used as a test of attentional/cognitive deficits in a wide variety of animal models of neurological disorders (Schizophrenia: Broberg, Dias, Glenthøj, & Olsen, 2008; ADHD: Cao, Yu, Wang, Wang, Yang, & Lei 2012; Huntington's: Sosti, Martínez-Horta, Pérez, Arenas, & Kulisevsky, 2014). Its design was based on the human ID/ED CANTAB task (Cambridge Cognition), which has also been modified for non-human primate use,

the extent to which comparable results are seen across species is limited only by the validity of the neurological model chosen. Similar patterns of results have been shown in both a PCP model of schizophrenia in rats and in Schizophrenic patients. For example, Rodefer, Murphy & Baxter (2005) found that sub-chronic PCP treatment in rats resulted in a significant impairment in the extradimensional shift stage, with rats needing significantly more trials to reach criterion than their saline treated counterparts. Similarly, Pantelis, Barber, Barnes, Nelson, Owen, & Robbins (1999) found that schizophrenic patients required significantly more trials to reach criterion at the extradimensional stage compared to age and IQ matched controls. However, the study also found deficits in intradimensional performance as compared to controls – an effect not seen in the Rodefer et al (2005) study. This however, could be due to subchronic doses used which may not have induced sufficient long-term perturbations of frontal function comparable to that of the Pantelis research where patients had an average length of illness of 27.5 years.

While examining set-shifting abilities in a PCP model of schizophrenia, Egerton, Reid, McKerchar, Morris, and Pratt (2005) found that deficits in the extradimensional shift were correlated with decreases in parvalbumin expression in the thalamic reticular nucleus. It has been suggested that the increased trials needed to reach criterion at the extradimensional shift for PCP treated animals was due to alterations in the ability of the thalamic reticular nucleus to mediate the relay of sensory information.

While the Petrof and Brown (2000) study implicated the thalamic reticular nucleus in a discrimination task that involved similar apparatus to that used in the

attentional set shifting task, their task relied on visual discriminations, and therefore the strategy adopted by the animals was not consistent with that used in set shifting. Transmission of olfactory sensory information differs from that of all other sensory modalities as information goes directly to cortex from the olfactory bulb without thalamic relay (Kay and Sherman, 2006). Given the olfactory component of the task, the rostral sector of the thalamic reticular nucleus is the logical target to produce a lesion while examining performing in the attentional set shifting task. The rostral sector of the thalamic reticular nucleus, often known as the cognitive sector, has dense reciprocal connections with the mediodorsal thalamus (Cornwall, Cooper, & Phillipson, 1990). The mediodorsal thalamus additionally has connections with the medial prefrontal cortex (Groenewegen, 1988). Birrell and Brown (2000) established that cell body lesions of the medial prefrontal cortex impair the shifting of attentional set.

Cross, Brown, Aggleton, and Warburton (2013) compared the effects of mediodorsal thalamus and medial prefrontal cortex lesions on object in place memory. It was found that both lesions resulted in impairments in recognition memory involving recency and associative information. Various other studies have also shown mediodorsal thalamus lesions causing impairments in tasks believed to be prefrontal dependent (Gaffan and Parker, 2000; Bailey and Mair, 2005; Mitchell, Browning and Baxter, 2007).

In those tasks with an attentional component, it is possible that connectivity with rostral thalamic reticular nucleus could explain some of the deficits in performance seen in prefrontal dependent tasks following mediodorsal thalamus lesions. Formation of attentional set requires the directing of attention towards one

stimulus dimension, while marginalising the processing of secondary irrelevant dimension. Given that both dimensions are presented simultaneously in a multidimensional form, the animal must successfully filter irrelevant information in order to direct their focus to that dimension currently being rewarded (Birrell & Brown, 2000; McAlonan & Brown, 2003)

Lesions of mediodorsal thalamus in other tasks of learning and attention have also revealed reversal deficits (Slotnick & Risser, 1990). Although these tasks have studied visuospatial attention, it has been seen that lesions of the mediodorsal thalamus result in significant reversal impairments. In addition to mediodorsal thalamus connections with medial prefrontal cortex, retrograde and anterograde tracing has shown cortical projections to mediodorsal thalamus originating from orbital prefrontal cortex (Négyessy, Hámori, and Bentivoglio, 1998).

Ibotenate lesions of orbital prefrontal cortex in rats were found to impair reversal learning in the attentional set shifting paradigm without affecting learning acquisition (McAlonan and Brown, 2003). A similar pattern of behaviour was more recently shown by Block, Dhanji, Thompson-Tardif, and Floresco (2007) following inactivation of mediodorsal thalamus using bilateral infusions of bupivacaine. It was found that mediodorsal inactivation disrupted strategy shifting in a cross maze from a response based strategy (making a 90° turn) to a visual based strategy, and vice versa. Acquisition of the initial strategy was unaffected by infusions. In mice, reducing activity in mediodorsal thalamus using DREADD increased perseverative errors in an operant reversal learning task, while leaving initial task acquisition unaffected (Parnaudeau, O'Neill,Bolkan. Ward, Abbas, Roth, Balsam, Gordon, and Kellendonk, 2013).

In primates, lesions of the magnocellular portion of the mediodorsal nucleus of the thalamus have been shown to impair reward based decision making. Using a crossed disconnection approach – orbital prefrontal cortex and amygdala lesions in one hemisphere and mediodorsal thalamus in the other - it was found that animals with mediodorsal thalamus lesions failed to change object selection in the face of reinforce devaluation. Animals with a nucleus accumbens lesion contralateral to the combined orbital prefrontal and amygdala lesions did not show this pattern of behaviour, demonstrating the importance of the orbital prefrontal-amygdala-mediodorsal thalamus circuit in cognition.

In light of the aforementioned results, it is anticipated that lesions of either mediodorsal thalamus or rostral thalamic reticular nucleus will result in significant impairments in performance on the attentional set shifting task. Given the centrality of this circuit to cognition, there are two possible outcomes – lesions could result in impairments in the shifting of attentional set, or in reversal learning. From the available data, it would be predicted that lesions of the rostral thalamic reticular nucleus would impair set-shifting, while mediodorsal lesions would be more likely to impair performance at reversal stages.

5.2 Methods

5.2.1 Animals

Twenty nine male Lister hooded rats (Charles River, UK) were used within this experiment. Throughout the study period rats were trained on daily 60 minute sessions between 08:00 and 17:00.

5.2.3 Apparatus

The test apparatus used in the attentional set-shifting paradigm was constructed from a large homecage. An acrylic panel was placed one third down the cage, which is itself divided into two sections of the same size. Digging bowls were placed in the two smaller compartments, with the larger section used as a free area for the rat to sit and consume its reward between trials. One small and one large removable acrylic panel were used to separate the rat from either or both sections, respectively. To grant access to the rat the panel was lifted from the slot.

The digging bowls were ceramic bowls (depth of 4cm and internal diameter of 7cm. The bowls were filled with various digging media which could be scented with various spices and herbs (See table 5.1). In each trial half a honey loop (Kellogg Company, UK) was buried 2cms below the surface of the medium in the centre of the bowl.

Pair	Odour	Medium
1	O1 Cinnamon, O2 Ginger	M1 Coarse tea, M2 Fine tea
2	O3 Sage, O4 Paprika	M3 Sand, M4 Grit
3	O5 Turmeric, O6 Clove	M5 Coarse sawdust, M6 Fine sawdust

Table 5.1 . A list of all odours and digging media used in the 7-stage attentional set-shifting task.

5.2.4 Attentional Set-Shifting Task

5.2.4.1 Habituation and Training

The day before training two ceramic bowls containing sawdust and six honey loops at the bottom, were placed in the home cage. All of the honey loops were consumed by the following morning.

During training rats were put in the waiting area section of the set shifting box. A ceramic bowl containing sawdust and half a honey loop placed on top was left in each of the two small sections. The large panel was removed, thus allowing the rat to explore both bowls and find and eat the reward. In the proceeding five trials the reward was placed progressively further from the surface of the sawdust until the rat was reliably digging to find the reward. During each trial, if the rat didn't find the reward within ten minutes the panel was placed down and a new trial was initiated with the timer set back to zero.

For the second stage of training, the rat performed a simple discrimination between two bowls filled with normal sawdust. One was scented with mint and the other oregano. The rats had to dig and learn which one was baited. The bowls were placed in the two small compartments, with only one scent paired with the reward. The side of placement was randomly determined for each trial, but there could be no

more than three instances where the correct bowl was on the same side. Rats were given up to ten minutes to uncover the reward, after which the trial was ended and the next trial initiated. If the rat first dug in the correct bowl, the latency from panel withdrawal and digging was written down and the trial was recorded as correct. If the rat first dug in the incorrect bowl, the latency from panel withdrawal and digging was written down and the trial was recorded as incorrect. If an incorrect response occurred within the first four trials of the stage, then the panel was placed over this compartment, and the rat was allowed to recover the reward from the correct bowl. After the first four trials however, access to both compartments were blocked off from the rat and a new trial was started. The order at which the rat approached the bowls was also noted down. The criterion for learning was six consecutive correct trials, which included the first four self-correct trials. Training took place no more than 3 days before testing.

5.4.2.2 Testing – 7-Stage task

The 7-stage attentional set shifting task was first described by Birrell & Brown (2000), comprising seven discrimination stages. The first stage was the simple discrimination (SD), in which rats were required to discriminate between two exemplars from a single dimension (odour or medium). A compound discrimination (CD) followed, in which the rewarded exemplar was the same from the previous stage, but a second irrelevant perceptual dimension was added. A reversals stage (Rev1) followed in which the stimuli from the compound discrimination remained, but the correct and incorrect responses were reversed (if cinnamon was correct and ginger incorrect in the CD, then ginger become the correct response). During

reversal stages animals are required to maintain attentional set pertaining to a specific dimension, as well as learn, and simultaneously unlearn, stimulus-reward associations. Subsequently there was an intradimensional shift, where all exemplars were replaced, but the attended perceptual dimension was the same. This was followed by a second reversal (Rev2) in which the correct and incorrect responses were again reversed. A final set of new exemplars was then introduced during which the previously incorrect dimension became rewarded – this stage was known as the extradimensional shift. The difference in trials to criterion between the ID and ED stages is referred to as the shift cost, and reflects the ability to totally shift set. It is expected that sifting set from one perceptual dimension to another is more difficult than shifting within a dimension. The final stage comprised a third reversal (Rev3). As with training, rats were required to achieve six correct consecutive trials to progress to the next stage, with the first four trials being nominated as "self-correct" trials (See table 5.2 for an exemplar procedure).

Stage	Discrminanda	Paired with
Simple discrimination	M1 (Coarse tea), not M2 (Fine tea)	No pairing
Compound discrimination	M1 (Coarse tea), not M2 (Fine tea)	O1 Cinnamon, or O2 Ginger
Reversal 1	M2 (Fine tea), not M1 (Coarse tea)	O1 Cinnamon, or O2 Ginger
Intradimensional discrimination	M3(Sand), not (M4 Grit)	O3 Sage, or O4 Paprika
Reversal 2	M4 (Grit), not M3 (Sand)	O3 Sage, or O4 Paprika
Extradimensional discrimination	O5 (Turmeric), not O6 (Clove)	M5 Coarse sawdust, or M6 Fine sawdust
Reversal 3	O6 (Clove), not O5 (Turmeric)	M5 Coarse sawdust, or M6 Fine sawdust

Table 5.2. An example of the stages and stimuli used in the 7-stage attentional set shifting task. The stages were always run in the same order, with the correct stimuli counterbalanced across rats.

5.2.3 Surgery

Ten rats received bilateral rostral thalamic reticular nucleus lesions with 0.2µl of 0.06M ibotenic acid at coordinates AP -1.4, ML ± 1.8, DV 5.9 (from dura) via pulled glass pipette as a bolus infusion. A further ten rats received 0.06M ibotenic acid lesions of mediodorsal thalamus at coordinates AP −2.8, ML ±0.8, DV −5.2 (from dura) via pulled glass pipette as a bolus infusion. Nine control animals received an injection with 0.2µl of 0.1M PB into mediodorsal thalamus at coordinates AP −2.8, ML ±0.8, DV −5.2 (from dura) via pulled glass pipette as a bolus infusion. Intraperitoneal injection of 0.25ml diazepam was administered after the animal was anaesthetised.

Given the extreme precision needed to successfully hit the thalamic reticular nucleus – due to its size and location – the decision was made to use only mediodorsal sham lesions for the control animals. Should the rostral thalamic

reticular nucleus have proven unsuccessful then there would have been a sufficient number of animals for an experiment in their own rights.

5.2.4 Immunohistochemistry

After perfusion, brain removal and preparation, tissue sections were stained for neuronal nuclei (see general methods for details).

5.3 Data Collection and Analysis

Data pertaining to trials to criterion, errors to criterion, dig latency and the number of non-dig trials was recorded. However, only trials to criterion and errors to criterion were analysed. It was not anticipated that either lesion would impair performance at the SD, CD and ID stages, a priori hypotheses were limited to impacts on performance at reversals, and an increased shift cost. Repeated measures ANOVAS were used to analyse performance with stage as the within subjects factor, and lesion group as the between subjects factor. Due to a priori predictions, separate RM ANOVAs compared both trials and errors to criterion across all 7 stages of the task, with additional ANOVAS being restricted to comparing performance across reversal stages, or between ID and ED stages.

5.4 Results

5.4.1 Histology

5.4.1.1 Rostral thalamic reticular nucleus lesion

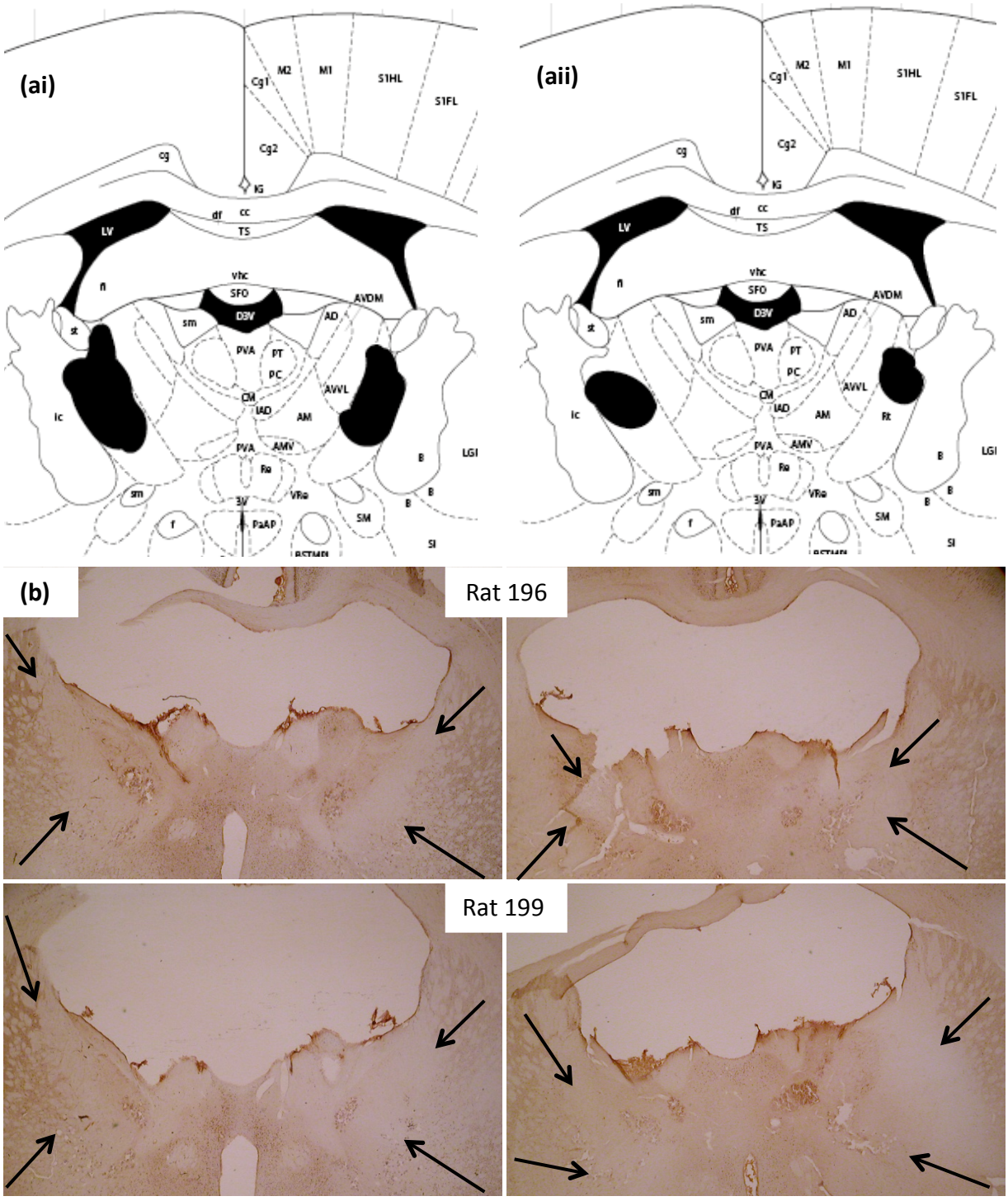


Figure 5.1. (a) Schematics representing the largest (ai) and smallest (aii) lesions of the rostral thalamic reticular nucleus. All rats had bilateral lesions of the thalamic reticular nucleus, (b) exemplar photographs of lesions from rats 196 and 199

5.4.1.2 Mediodorsal thalamus lesions

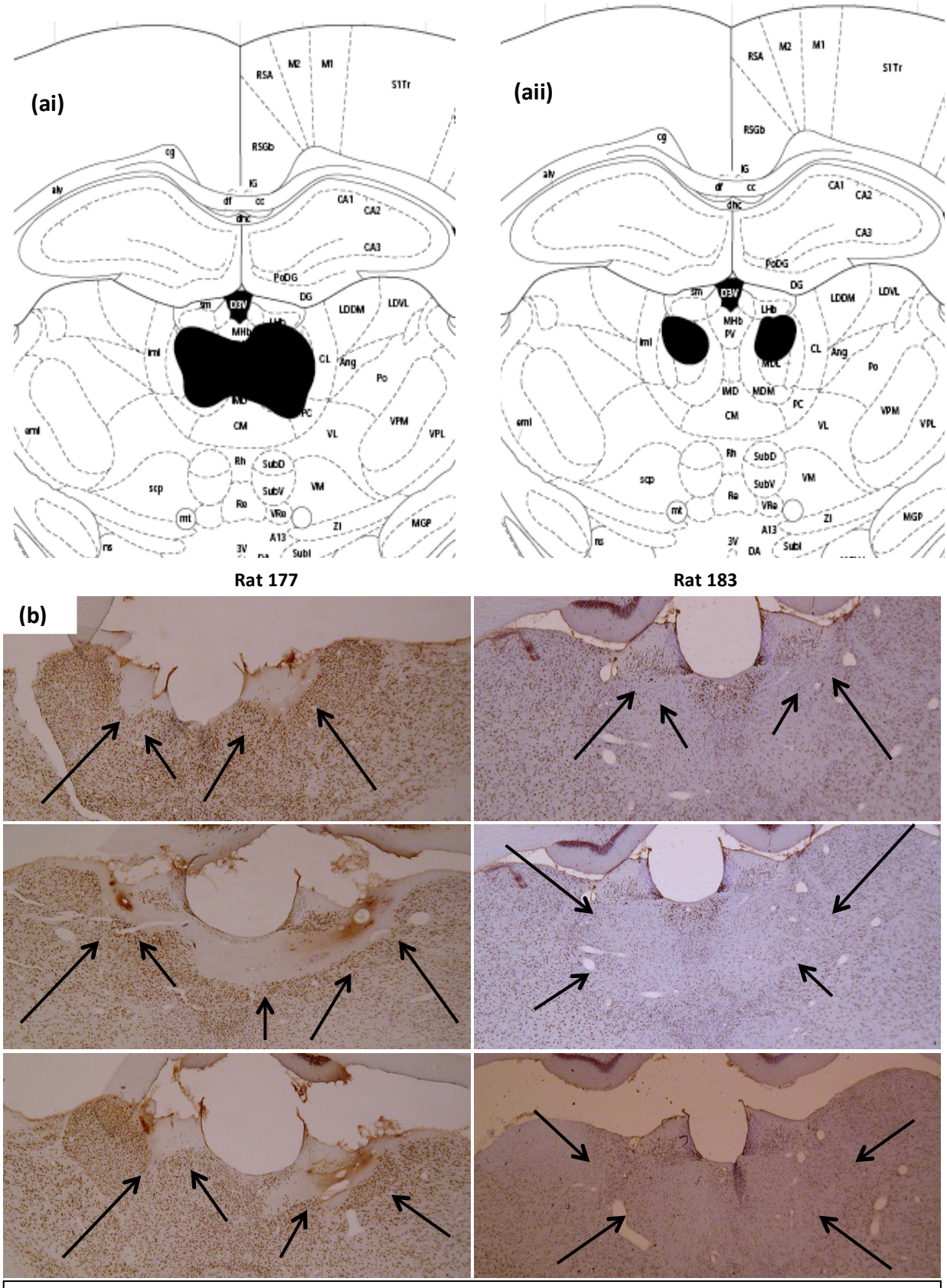


Figure 5.2. (a) schematics representing the largest (ai) and smallest (aii) lesions of mediodorsal thalamus. All rats had bilateral lesions of the mediodorsal thalamus, (b) exemplar photographs of ibotenic acid lesions from rat 177 and control lesion from rat 183.

5.4.2 Trials to criterion

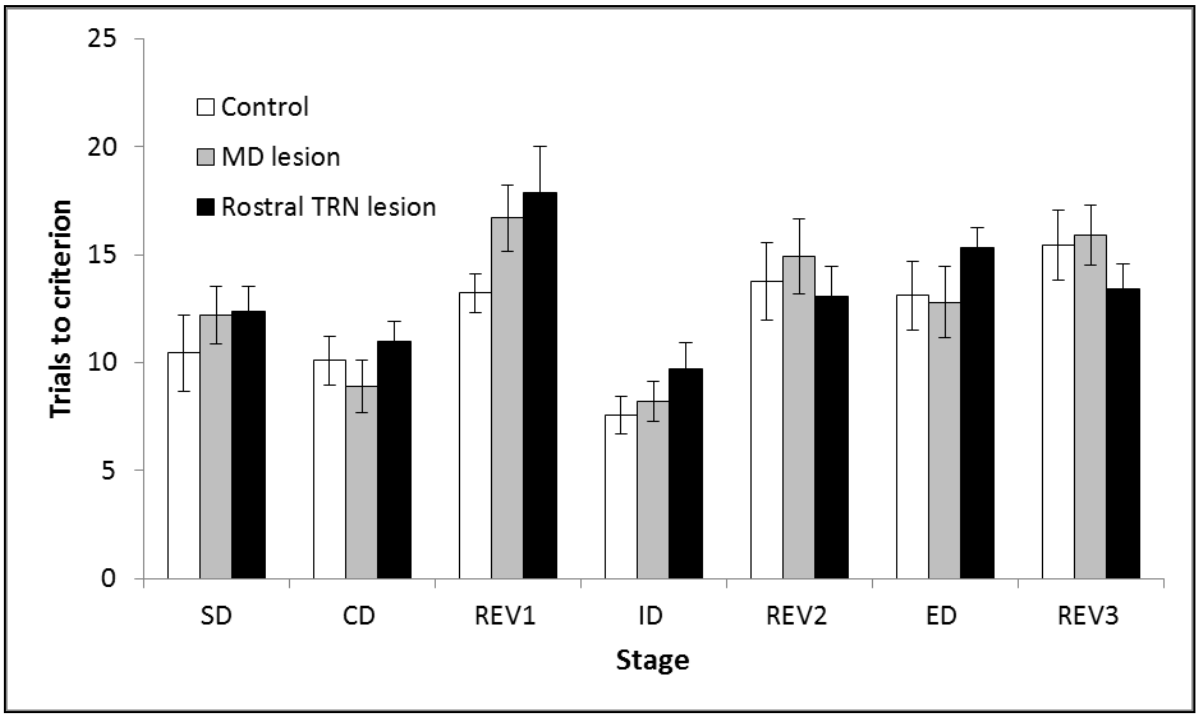


Figure 5.3. Trials required to reach criterion in the 7 stage task. Performance in every stage of the task showed a similar pattern to control performance and did not significantly differ by lesion group.

Performance of rats with either mediodorsal thalamus or rostral thalamic reticular nucleus lesions did not differ to that of rats with control lesions. All rats needed more trials to reach criterion across the three reversal stages, but there was no significant effect of lesion type on trials required (significant main effect of stage [$F(6,156)=11.47$, $p<.01$] and no stage*group interaction [$F(12,156)=.94$, n.s.]). Additionally, all rats showed a shift cost, whereby they required significantly more trials to reach criterion at the extradimensional stage as compared to the intradimensional stage (See figure5.3), but the shift cost did not differ according to lesion group. An ANOVA restricted to ID and ED revealed a significant main effect of stage [$F(1,26)=25.64$, $p<.01$] but no stage*group interaction [$F(2,26)=.101$, n.s.]. The

significantly greater number of trials required to reach criterion at the extradimensional stage demonstrates that all groups successfully formed an attentional set. There was also no impairment in reversal learning (ANOVA restricted to reversal stages showed a non-significant main effect of stage [$F(2,52)=1.21$, $p>.05$] and no stage*group interaction [$F(4,52)=1.24$, n.s.].

5.4.3 Errors to criterion

Errors reflected the total trials to criterion and did not differ by lesion group. The number of errors made differed as a function of stage, but there was no interaction with lesion (significant main effect of stage [$F(6,156)=18.73$, $p<.01$] but no stage*group interaction [$F(12,156)=1.38$, n.s.]).. The most errors occurred during the reversal stages, but the lesion type did not influence error rates. ANOVA restricted to reversal stages showed no significant difference in trials to criterion as a function of lesion (non-significant main effect of stage [$F(2,52)=1.92$, $p>.05$] and stage*lesion interaction [$F(4,52)=1.43$, $p>.05$]. Additionally, all made significantly more errors during the extradimensional stage compared with intradimensional stage, reflecting a cost of shifting attention from one stimulus dimension to another: ANOVA restricted to ID and ED revealed a significant main effect of stage [$F(1,26)=13.595$, $p<.01$] but no stage*lesion interaction [$F(2,26)=.105$, $p>.05$]. (See figure 5.4).

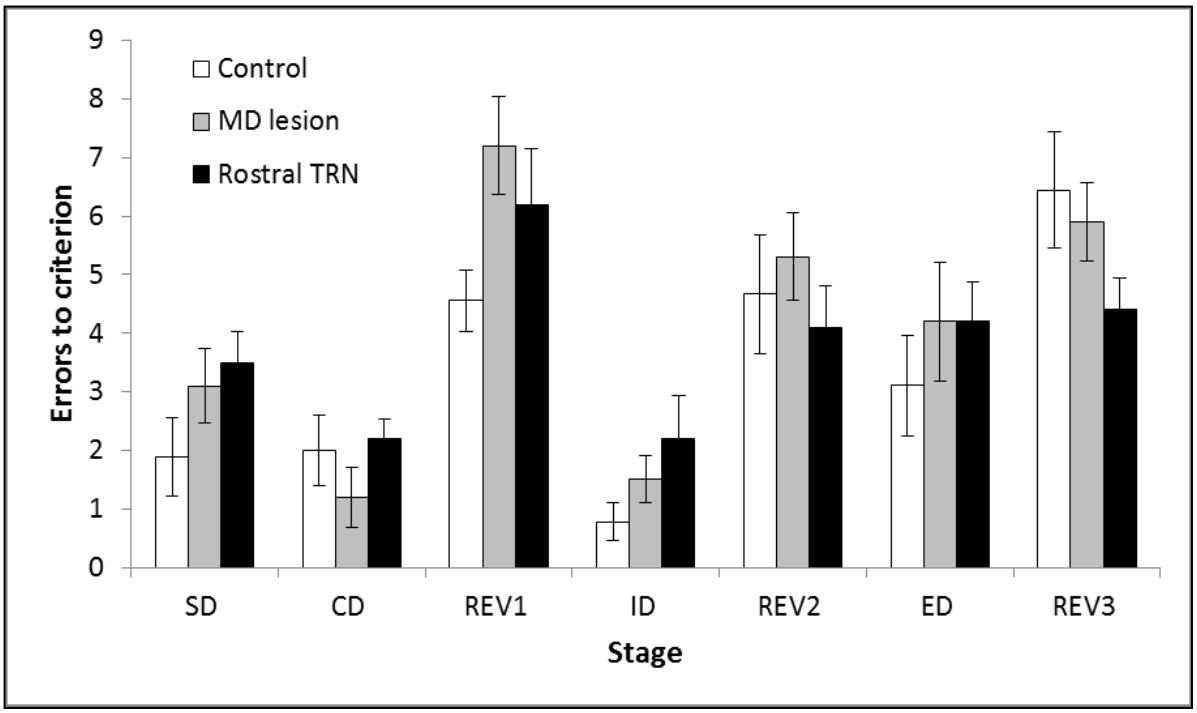


Figure 5.4. Number of errors made in each stage of the 7 stage task. Performance across the stages did not differ between lesion and control animals.

5.5 Discussion

This chapter aimed to examine the effects of bilateral ibotenic acid lesions of mediodorsal thalamus and rostral thalamic reticular nucleus on performance in the attentional set shifting task. Given the sensitivity of the task to deficits induced by prefrontal cortex lesions in rats (Birrell and Brown, 2000; McAlonan and Brown, 2003), and the strong connections between both mediodorsal thalamus and rostral thalamic reticular nucleus with prefrontal cortex, we expected to see similar impairments. Reports from previous literature have shown that deficits in performance on prefrontal dependent tasks can be induced by mediodorsal thalamus lesions, although the majority of these studies focused on working memory tasks.

5.5.1 Mediodorsal thalamus

As with numerous experiments it was found that mediodorsal thalamus lesions did not disrupt basic acquisition of the task (Chudasama, Bussey & Muir, 2001; Block et al, 2007; Parnaudeau et al, 2013). Mediodorsal thalamus animal performance did not significantly differ from controls on any of the compound discrimination stages. However, inconsistent with these studies was the finding that the mediodorsal thalamus lesions did not impair performance on reversals. Chudasama, Bussey and Muir (2001) examined reversal learning on a visual discrimination task in rats following excitotoxic lesions of the mediodorsal thalamus. They revealed that rather than affecting response inhibition in the early trials of the reversal stage, the lesion affected the ability of the rat to acquire the new stimulus-reward association. Rats made numerous non-perseverative errors in the later

stages of the reversal. A non-perseverative error is an error made that suggests the animal is not using a previously employed rule, but is nonetheless incorrect. In this experiment, it suggested that the animal was struggling to learn the new stimulus-reward association.

Our experimental paradigm does not permit the same kind of analysis of reversal performance as that in the Chudasama, Bussey and Muir (2001) paper given our six in a row correct criterion, and the low number of trials per stage. The number of trials taken to reach criterion on the reversal stages was approximately 15 trials, and was completed in a single session whereas Chudasama, Bussey and Muir (2001) reported ~1200 errors to reach 85% criterion on their third reversal stage.

Comparisons between operant and non-operant tasks of discriminations can be difficult given the vast differences in information obtainable from trial data. Our task has revealed itself to be sensitive to significant impairments in reversal learning as demonstrated by McAlonan & Brown (2003) with orbtital prefrontal cortex lesions. It has also shown itself to be sensitive to deficits in attentional set shifting (Birrell & Brown, 2000). However, it is possible that the mediodorsal thalamus lesion produced only a weak effect that is not seen when the task is as easy for the rat as the bowl-digging discrimination. Indeed, although mediodorsal thalamus projects to orbital prefrontal cortex and orbital prefrontal cortex lesions produce strong behavioural impairments in the attentional set shifting task, this does not mean that mediodorsal thalamus lesions would or should produce as strong an effect. Connectivity between regions does not mean that perturbing function of either region will result in the same behavioural consequences.

For example, Cross et al (2013) found that while medial prefrontal cortex lesioned animals performed significantly worse than controls at both 5 minute and 3 hour delays in an object in place task, mediodorsal thalamus lesioned animals only failed to discriminate between stationary and moved objects at the 5 minute delay. Therefore, while the mediodorsal thalamus lesion produced behavioural effects that were similar to those produced by medial prefrontal cortex lesions, they were not as severe.

Differential results from other studies can be used to explain our results. In two distinct experiments, the effects of prefrontal and mediodorsal thalamus excitotoxic lesions were examined using the 5-choice serial reaction time task. These studies offer more of a comparison with our task given that they examine aspects of attention as compared to the majority of studies that focus on mediodorsal-prefrontal connections within the remit of working memory function.

The first experiment showed that lesions of mediodorsal thalamus resulted in significantly more premature responses, with perseverative errors remaining unaffected in trials with long inter trial intervals (Chudasama, Bussey, and Muir, 2001). Comparably, prefrontal lesions also increased the number of premature responses. However, unlike the mediodorsal thalamus lesions there was a significant increase in the number of perseverative errors made (Chudasama, Passetti, Rhodes, Lopian, Desai, and Robbins, 2003).

Our task does not have a period in which responses are considered premature as the rat is not required to wait for a fixed amount of time once the panel is withdrawn. Furthermore, while we can collect response latencies, they are unreliable as the rats differ in their behaviour after they retrieve a reward – some

will consume it at the back of the box while others sit right by the panel. We do not wait for rats to approach a predetermined location before we lift the panel, rather we wait until the reward has been fully consumed. Without controlling for the location of the animal prior to revealing the bowls, we cannot examine response latencies with any degree of reliability.

5.5.2 Rostral thalamic reticular nucleus

Lesions of the rostral thalamic reticular nucleus were found to not impair the shifting of attentional set. It had been predicted that the thalamic reticular nucleus could be involved in the filtering of irrelevant sensory information, and therefore promote the formation of attentional set. By perturbing thalamic reticular activity, it was believed that the shifting of attentional set would be impaired, resulting in greater trials to criterion required at the extradimensional stage. The greater number of trials required would reflect a shift cost, and the difficulty in attention being 'disengaged' from one stimulus dimension and redirected toward another.

It appears that despite connectivity with regions known to be involved in the shifting of attentional set, the rostral thalamic reticular nucleus is does not appear to be an important functional element of this cognitive circuit.

Similar findings were documented by Wilton, Baird, Muir, and Aggleton (2001). In addition to connections with mediodorsal thalamus, the rostal sector of the thalamic reticular nucleus also has strong connections with anterior thalamus (Gonzalo-Ruiz, & Lieberman, 1995; Lozsadi, 1995). The anterior thalamus has been widely implicated in spatial memory, with lesions of anterior nuclei resulting in significant impairments (Aggleton, Hunt, Nagle, & Neave, 1996; Dumont, Amin, &

Aggleton, 2014; Loukavenko, Ottley, Moran, Wolff,& Dalrymple-Alford, 2007). It was hypothesised that by virtue of this connectivity that lesioning the rostral thalamic reticular nucleus would impair spatial memory. However, Wilton, Baird, Muir, and Aggleton (2001) found that lesions of the rostral thalamic reticular nucleus did not result in significant impairments in spatial memory. All tasks used (t-maze, morris water maze, and radial maze) have all been shown to be sensitive to anterior thalamus lesions, but no effect on spatial learning on memory was found following rostral thalamic reticular nucleus lesions.

This chapter examined the effect of lesions of the cognitive sector of the thalamic reticular nucleus on attentional set shifting. The formation of attentional set is a complex behaviour, with an inability to form and shift attentional set underpinning many cognitive issues seen in several psychological disorders. This study focused only on the small rostral sector of the thalamic reticular nucleus as it was hypothesised that this area was most likely to be involved in performance in such a task. Unlike the sensory sectors of the thalamic reticular nucleus which have specific projection (visual TRN to LGN for example), the rostral sector has more diffuse, less specific projections. Perhaps the rostral sector is more involved in general consciousness/arousal rather than a specific cognitive function.

Given the lack of behavioural studies examining lesions to specific thalamic reticular nucleus sectors, it is prudent to study the effects of more global perturbations to function. Reducing neurotransmitter input into the thalamic reticular nucleus offers a subtle approach to study its contribution to attention while also examining the role of a specific system. Following suggestions that the thalamic reticular nucleus is involved in schizophrenia and the established link between

dopamine and schizophrenia, reducing dopamine input into the area is a strong candidate for further examination. The final experimental chapter examines the role of dopamine depletion in the thalamic reticular on performance in a two-alternative forced choice reaction time task. More specifically, it will aim to study the role of the thalamic reticular nucleus in orienting and re-orienting of attention.

6-hydroxydopamine lesions of the thalamic reticular nucleus impair the re-orientation of attention in a two-alternative forced choice reaction time task

Abstract

Due to the strategic position of the thalamic reticular nucleus, and its connectivity with both thalamus and cortex, it is believed to play a pivotal role in visuospatial attention. Cell body lesions of the TRN have been shown to impair the orienting of attention by abolishing the usefulness of valid cues when directing attention. Widespread dopamine projections throughout the brain are additionally implicated in visuospatial attention. Tracing studies have revealed that the TRN receives dopaminergic innervation originating from the substantia nigra pars compacta. Striatal dopamine depletion does not impair attentional orienting in the rat, but we hypothesised that dopamine depletion in the TRN could impair attention.

Rats were trained to perform a two-alternative forced choice reaction time task. In order to induce endogenous orienting of attention, the spatial probability of the target was manipulated across five foreperiods so that left targets were more probable at earlier foreperiods, and right targets at later foreperiods. One group of rats received bilateral injections of 6-hydroxydopamine into the thalamic reticular nucleus, with a second group receiving sham lesions; the cannula lowered to injection site, but without infusion.

Before surgery, reaction time to targets was found to be faster to more probable locations. After bilateral lesions rats showed no overall impairment in reaction time, but showed a slowing in the re-orientation of attention. The rats maintained the initial reaction time advantage for left (more probable) targets even when right side targets became more probable. This suggests that dopamine in the TRN is involved in the re-orientation of attention. These results have significant implications for understanding disorders that manifest aberrant dopaminergic function and cognitive impairments, such as schizophrenia and Parkinson's disease.

6.1 Introduction

An inability to disengage attention from a once pertinent stimulus leads to rigidity which can retard learning new contingencies, while an inability to maintain attention will similarly interfere with learning. Many psychological disorders have a key attentional component, demonstrating just how critical a functional attention system is to our ability to interact with our environment (Dujardin, Tard,Duhamel, Delval,Moreau,Devos, & Defebvre, 2013;. Rock, Roiser, Riedel,& Blackwell, 2014; Nuechterlein et al, 2015; Maurage et al, 2017). In an extended framework of executive function it is clear that attention contributes significantly; understanding the intricacies of attentional processes should therefore be a critical endeavour.

Previous work relying upon cell body lesions suggests a pivotal role for the thalamic reticular nucleus in spatial orienting of visual attention (Weese et al, 1999). However, destroying the cell bodies removes not only the feedback to thalamus, but also removes the modulatory input of particular neurotransmitter systems projecting onto these cell bodies. The complexity of connections throughout the attentional systems means that several major neurotransmitter systems (dopamine, acetylcholine, noradrenaline) have been implicated in different forms of attention, including spatial orienting. Attempting to understand attention in its entirety is only feasible by picking apart the contributions of each individual system.

A vital element of an animal's cognition is its ability to simultaneously attend to relevant, while ignoring irrelevant, information. Once salience of cues within the environment has been determined there must be active maintenance of attention and, when necessary, re-orientation of attention. This re-orientation of attention to

some extent reflects cognitive flexibility and the ability to utilise changing information to re-direct focus to more relevant targets. Dopamine has been implicated in the latter process of re-orientation of attention (Kähkönen, Ahveninen, Pekkonen, Kaakkola, HuttunenIlmoniemi, & Jääskeläinen, 2002; Nieoullon, 2002.).

Dopamine agonists have been shown to impair the shifting of attention in a task of attention using a radial arm maze (Ragozzino, Detrick, & Kesner, 1999; Floresco, Magyar, Ghods-Sharifi, Vexelman, & Maric, 2006). Termed set-shifting by the authors, this task differs somewhat from the attentional set shifting task described in chapter 4, as it requires a change in the response rule, rather than a shift of attention to new stimulus features. Therefore here the term 'switching' will be used to refer to these kinds of task. The rat is placed in a maze and in the first stage has to make a 90° turn to receive a food reward. The task shift then requires the animal to enter the arm with the visual cue of black and white stripes – the switch is therefore from an egocentric response-based rule to a visual stimulus-based rule. Floresco, Magyar, Ghods-Sharifi, Vexelman, & Maric, (2006). examined the effects of infusions of multiple dopamine receptor agonists and antagonists into the prefrontal cortex on performance in the aforementioned task. They showed that the number of trials required to reach criterion varied as a function of drug type. The dopamine D4 agonist PD-168,077 and the D2 antagonist eticlopride were shown to impair switching from response to visual cues, specifically by increasing perseverative errors. Conversely, the D4 receptor antagonist was shown to improve switching abilities. The relationship between an attentional set-shifting task (e.g., Birrell & Brown's 2000 task) and a response rule switching task is not clear, but nonetheless this data suggests that there might be an important dopaminergic

contribution to attention, and more specifically the shifting/re-orientation of attention.

Immunohistochemical techniques have shown that the thalamic reticular nucleus is innervated by dopaminergic axons originating in the substantia nigra pars compacta (Anaya-Martinez, Martinez-Marcos, Martinez-Fong, Aceves, & Erlij, 2006; Freeman, Ciliax, Bakay, Daley, Miller, Keating, Levey, and Rye, 2001). These methods often utilise chemical markers for tyrosine hydroxylase. Tyrosine hydroxylase is the first enzyme involved in the synthesis of catecholamines including dopamine, by catalysing the conversion of L-tyrosine into L-DOPA. This involvement therefore makes it ideal to be used as a marker for catecholamine neurons, thus allowing for their localisation throughout the brain. Using the aforementioned technique Freeman and colleagues (2001) showed in rhesus monkeys and Sprague-Dawley rats, that tyrosine hydroxylase immunoreactive terminals were widely distributed throughout the thalamus including principal sensory relay nuclei and the thalamic reticular nucleus.

The thalamic reticular nucleus is rich in dopamine D4 receptors (Mrzljak, Bergson, Pappy, Huff, Levenson, & Goldman-Rakic, 1996; Khan, Gutiérrez, Martin, Penafiel, Riviera, & De La Calle, 1998). This is especially significant from a cognitive and pathological standpoint given the actions of the atypical antipsychotic clozapine. Clozapine has been shown to have a high affinity for the D4 receptor; 10-fold higher for D4 as compared to D2 receptors (Sanyal & Van Tol, 1991). Clozapine has been shown to be effective in the treatment of those patients who fail to respond to traditional antipsychotics (Meltzer, 2010). Seemingly unique to clozapine is its

capacity to evoke significant positive change within the cognitive domain (Spagna, Dong, Mackie, Li, Harvey, Tian, Wang, and Fan, 2015). In a review and meta-analysis, Keefe, Silva, Perkins & Lieberman (1999) indicated support for cognitive improvements following treatment with clozapine. This was confirmed by another review by Meltzer and McGurk (1999), who noted significant improvements in attention and verbal fluency. More recent studies also confirm the efficacy of clozapine to improve cognitive function in both animal models and patients (Li, Wu, & Li, 2007; Hill, Bishop, Palumbo, & Sweeney, 2011). The role of the thalamic reticular nucleus in attention, the presence of D4 receptors, and clozapine's ability to improve cognitive symptoms in ways not seen in other antipsychotics with different dopamine receptor blockade(s) presents an interesting line of investigation. Taken together, it strongly suggests that dopamine innervation of the thalamic reticular nucleus is involved in attention.

Thus far, the behavioural role of dopamine in thalamic reticular function, and more specifically attentional processes, is unknown. Although pharmacological evidence points for a role of the thalamic reticular nucleus in filtering incoming sensory information and dopamine is implicated re-orienting attention, the exact role of dopamine in the thalamic reticular nucleus is unclear. In a task of endogenous orienting of attention, it is predicted that depleting dopamine in the thalamic reticular nucleus will result in impairments in the re-orientation of attention, while leaving the initial orienting of attention in-tact.

6.2 Methods

6.2.1 Animals

Twelve male Lister hooded rats (Charles River, UK) were used within this experiment. Throughout the study period rats were trained on daily 45 minute sessions between 12:00 and 18:00.

6.2.2 Apparatus

The 9-hole operant box (Paul Fray, UK), described previously by Carli, Robbins, Evenden & Everitt (1983) and Brown & Robbins (1991), was employed during these series of experiments. The operant box comprises an array of 9 poke holes, although only the central three were used during this experiment. Each hole has a white LED stimulus light at the rear, as well as a photoeletric cell at the entrance which serves to detect breaks in a vertical infrared beam. Each correct response resulted in a reward pellet (45mg TestDiet, Richmond, IN, USA) being delivered to a food hopper containing a light, occluded by a vertical hinged panel. The hopper was positioned on the opposite wall to the response holes. The rat must approach the hopper and depress the panel in order to reach the reward pellet. A house light (3 watt bulb) and speaker were positioned on the ceiling of the chamber. The entire testing chamber was housed within a wooden sound-attenuating box with a fan that provided both ventilation and background white noise.

6.2.3 Spatiotemporal Target Probability Signal Reaction Time Task

6.2.3.1 Habituation

Step 1 – Habituation to food hopper. The first day of habituation involved placing a

small amount of food pellets in the hopper and leaving the rat for 20 minutes to ensure that all of the pellets were eaten. If there were remaining pellets they were left for another 10 minutes. All rats completed this stage in a single session.

Step 2 – Fixed ratio 1 schedule. For the second stage of habituation, animals were rewarded for single presses of the food hopper panel. All rats completed the desired 100 hits within a single testing session.

Step 3 – Poke training habituation to 600ms. The third stage required the animals to poke their nose in the central response hole where they were rewarded with a food pellet delivered to the hopper. The hole was lit until the rat made the nose-poke, at which point the light went off and the rat had to maintain the nose poke until they heard a tone. If the rat removed its nose before the specified time period the light went off and they were required to tap the food hopper again, after which the central light came back on. After correct responses, when the rat collected their reward the central light was lit again, signalling for them to poke their nose again. The duration of the nose poke began at 100ms and, after the desired number of correct responses, progressed to 200, 300, 400, 500 and finally 600ms. All rats were trained in this stage within 14 days.

Step 4 – Reaction time 1, habituation to flanker holes. In the final stage of habituation the rat was rewarded for attending to flanker lights (immediately to the right or left of the central hole). When the light came on the rat had to hold their nose in the central hole, after which a tone sounded and a flanker light came on. The animal then had to hold its nose in the side hole to receive the reward. At first the animal had 10 seconds to place its nose in the required side hole which decreased to 3 seconds and finally 1 second – movement to the next stage occurred when they

made at least 70 correct responses within a single session. Once the rat could perform satisfactorily with a maximum response latency of 1 second, it was ready to start the actual task. All rats reached criterion within 16 days.

6.2.3.2 Experimental testing

The animal was required to make a nose poke in the central hole for a variable period (200, 300, 400, 500, or 600ms). The end of this foreperiod was defined by the central light going off, a continuous tone being presented, and the onset of a flanker light either to the right or the left. The tone ceased when the animal withdrew its nose from the central hole. The rat was then required to make a response by poking its nose in the same hole as the target (flanker) light. The target light remained illuminated until the animal responded, or a late response was measured (not responding after 10 seconds from nose removal). To induce endogenous orienting of attention, the spatial probability of the target was varied as a function of foreperiod length (see figure 6.1). The right:left ratio of target onset as a function of foreperiod was as follows: 8:92 at 200ms; 33:66 at 300ms; 50:50 at 400ms; 66:33 at 5ooms and 92:8 at 600ms. Therefore, at earlier foreperiods it was more likely that the target would appear on the left, and at later foreperiods it was more likely that the target would be presented on the right.

To prevent side bias developing, a correction procedure was used whereby incorrect trials were repeated until correct response was obtained. If the animal removed its nose before the cessation of the foreperiod then an anticipatory response was recorded and there was a 1 second time out period initiated. No reward was given if the animal made late, anticipatory or incorrect responses and

there was a one second time out period. For each of these responses they had to

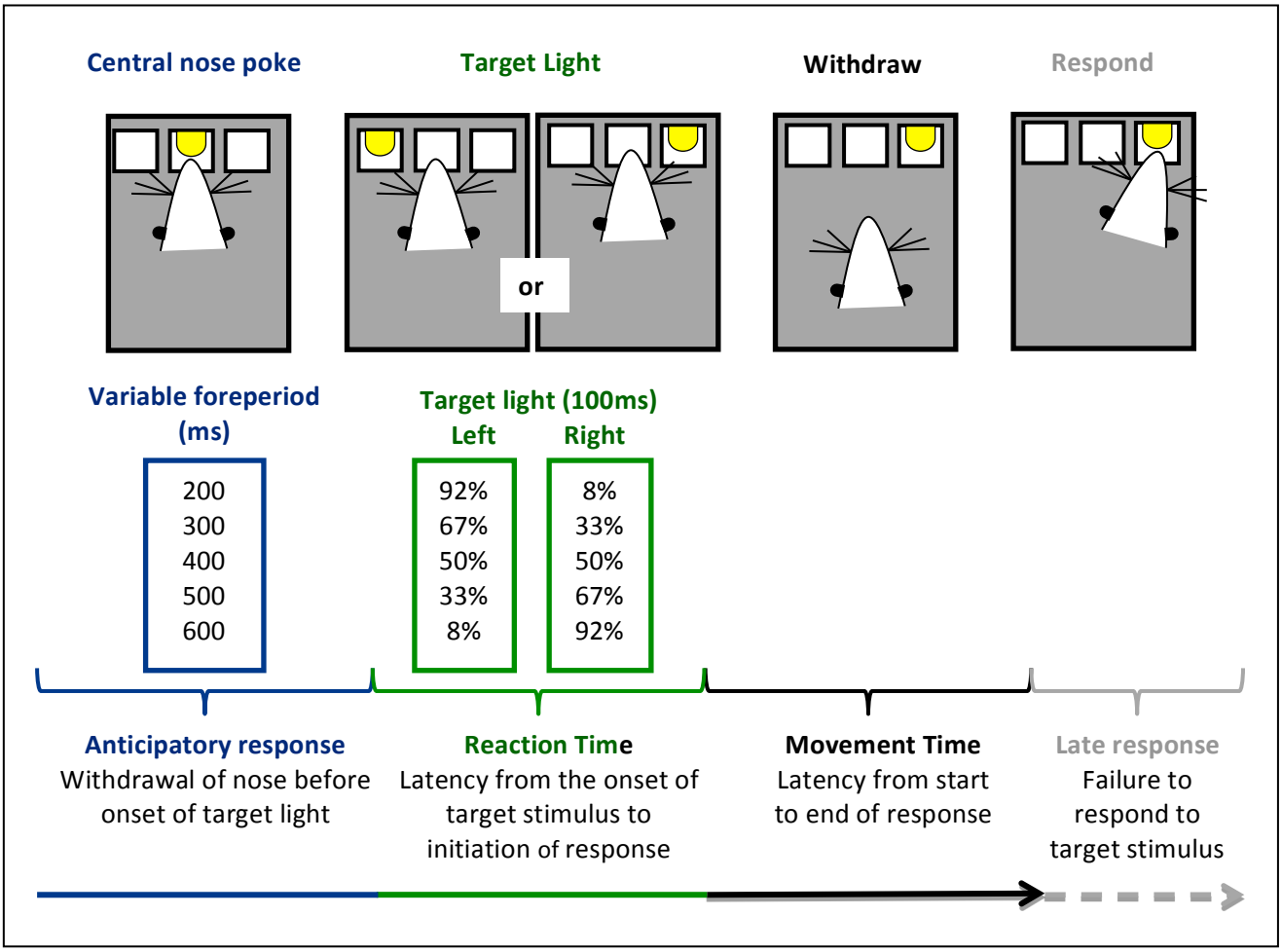


Figure 6.1. Schematic depicting a single trial in the Spatiotemporal Target Probability Signal Reaction Time Task.

initiate a new trial by pushing the panel to the food tray.

6.2.4 Surgery

Rats were intraperitoneally injected with desipramine (25ug/kg injected at 12.5ug/ml); Sigma chemicals), a noradrenergic reuptake inhibitor 5 minutes before surgery, and at least 30 minutes before the injection of 6OHDA.

Eight rats received unilteral injections of 0.1µL 6OHDA (15µg/µl) kept in the freezer until use to reduce risk of oxidation) using a 0.5 µl round tip Hamilton syringe in the thalamic reticular nucleus at coordinates AP -2.3mm; ML ± 3.4mm; DV -4.6mm

(from dura) (Paxinos & Watson, 1986). The toxin was injected over 10 minutes; the needle being left in situ for 5 minutes.

Four control rats received unilateral insertions without vehicle; the needle was lowered to the site of injection at coordinates AP -2.3mm; ML ± 3.4mm; DV -4.6mm (from dura) (Paxinos & Watson, 1986). The 0.5 µl round tip Hamilton syringe was left in situ for 5 minutes with no infusion. The decision was made not to inject a vehicle as it has been shown to reduce dopamine.

Counterbalanced unilateral lesions were made initially, and several weeks after the first surgery a second lesion (therefore resulting in bilateral lesions) was made using the same procedure. A unilateral, followed by a bilateral, lesion approach was chosen due to the use of spatial probabilities in the task. A two stage approach would allow for a comparison between the effects of lesion side on reaction time to both left and right targets.

Rats were given at least 3-4 days to recover after each surgery before testing commenced.

6.2.4.1 Immunohistochemistry

After perfusion, brain removal and preparation tissue sections were stained for tyrosine hydroxylase (see general methods for details).

6.3 Data Collection and Analysis

Behavioural measures available were reaction time, movement time, and response side. From this, percentage correct, late and anticipatory could also be calculated. Reaction time data was analysed both as absolute values, and also as the calculated reaction time difference. The reaction time difference value represents the difference in reaction time between the most and least probable stimulus location (most minus least). Thus, a negative value indicates a quicker reaction time to more probable locations.

Movement time data was left in its average form for both right and left targets across the five delays.

Individual repeated measures ANOVAS with surgery (2: pre or post) and reaction time difference as a function of delay (5: 200, 300, 400, 500, or 600ms) as within subjects factors, and group (control or lesion) as the between subjects factors were conducted to analyse reaction time and movement time differences following unilateral and bilateral surgery. Reaction time is defined as the latency from the onset of target stimulus to the initiation of a response, and movement time is the latency from the start to the end of a response.

Delay	Reaction time difference
200	Left – right
300	Left – right
400	Left – right
500	Right – left
600	Right – left

Table 6.1. Reaction time difference calculations.

6.4 Results

6.4.1 Immunohistochemistry

Immunohistochemical analysis using tyrosine hydroxylase immunoreactivity revealed that all lesion animals sustained bilateral dopamine depleting lesions. A reduction in the number of tyrosine hydroxylase positive neurons in the substantia nigra pars compacta revealed a reduction of up to 68% in the area as compared with control animals (see figures 6.2 and 6.3).

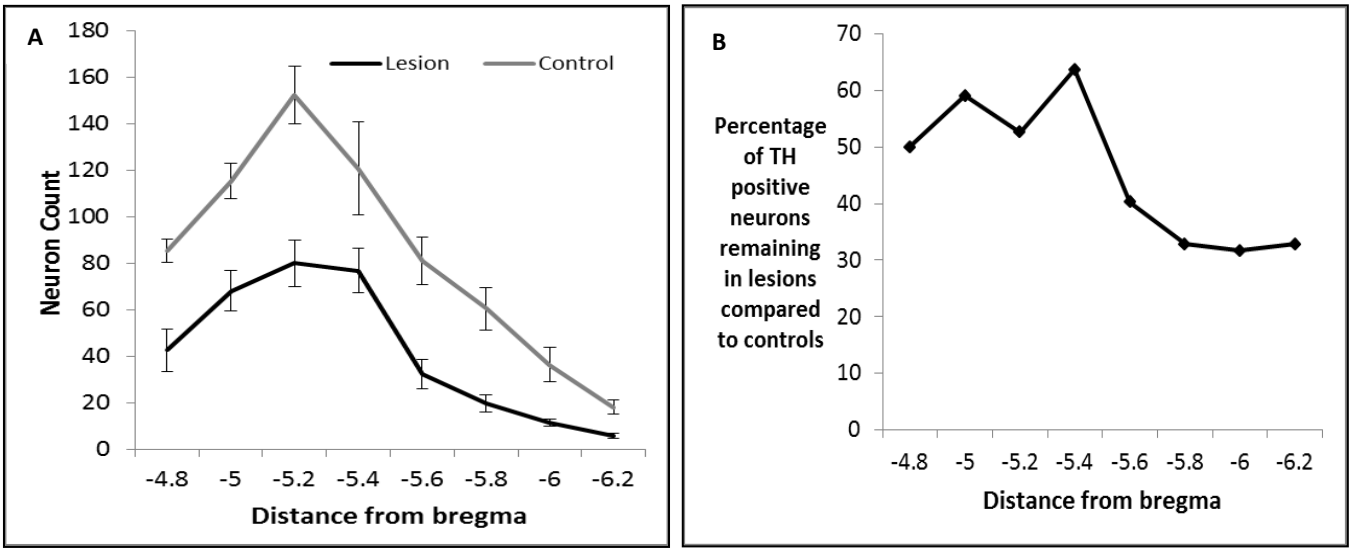


Figure 6.2. (A) Neuron counts in the substantia nigra pars compacta of lesion and control animals (B) percentage of tyrosine hydroxylase positive neurons remaining in lesion animals compared to control animals.

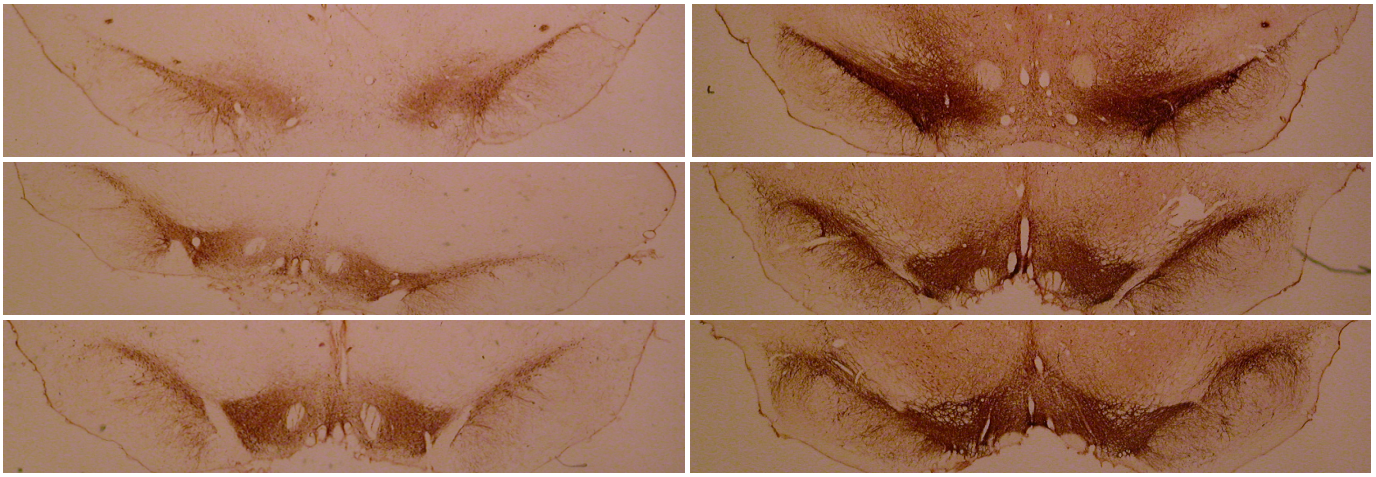


Figure 6.3 . Examples of the substantia nigra pars compacta following (a) bilateral 6hydroxydopamine lesions [rat 188] and (b) bilateral control lesion [rat 194].

6.4.2 Movement Time

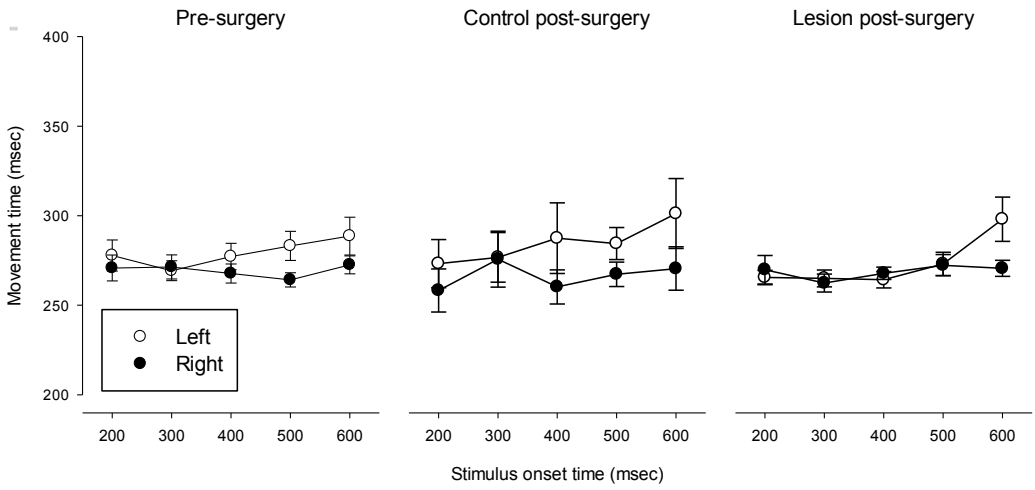


Figure 6.4. Line graphs depicting movement time to right and left stimuli : left panel pre-surgery performance, middle panel post surgery sham, right panel post surgery lesion.

Figure 6.4 shows the movement time for correct responses pre-surgery (left panel), and the control sham-surgery (middle panel) and lesion (right panel) groups. Similar to the pattern of results for percentage of correct responses, the pattern of movement times reflected the stimulus probabilities, particularly at the longest foreperiod where movement time was significantly slower for correct responses to the less probable stimulus (Side x Foreperiod: $F(4,40) = 4.5$, $p < 0.01$, $\eta_p^2 = 0.31$, Observed power = 0.91). Furthermore, this pattern was similar before and after surgery for both the lesion and the control group, with no interactions between surgery and group and any other factor (all F-ratios < 1.0).

6.4.3 Percentage of correct responses

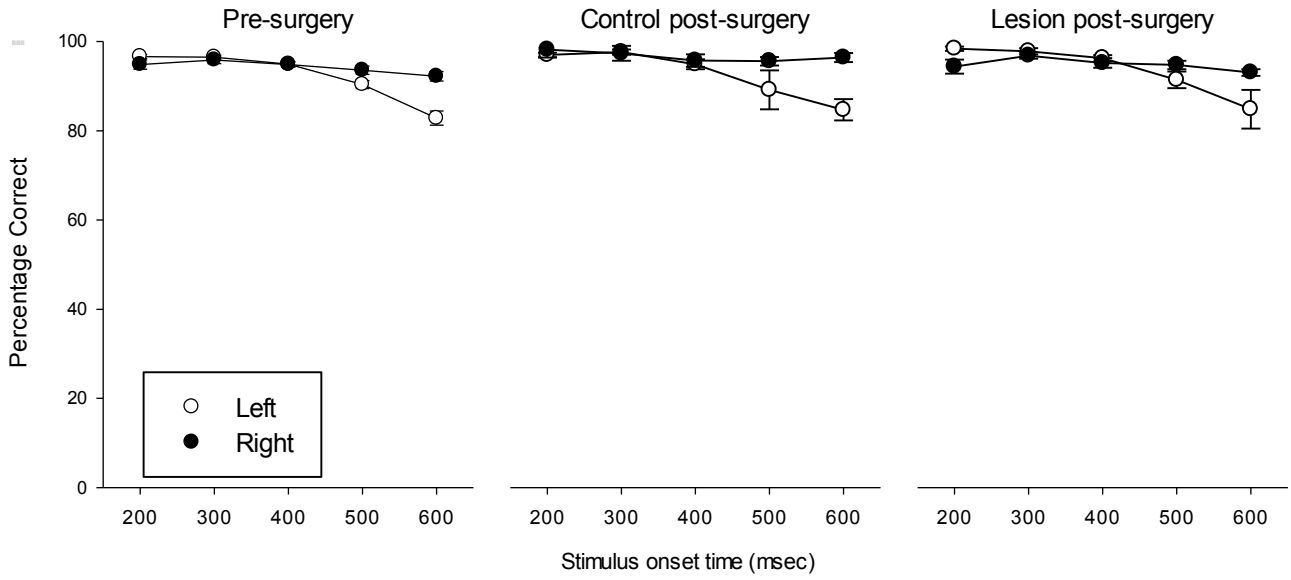


Figure 6.5. Line graphs depicting percentage of correct responses to right and left stimuli : left panel pre-surgery performance, middle panel post surgery sham, right panel post surgery lesion

Figure 6.5 shows the percentage of correct responses pre-surgery (left panel), and the control sham-surgery (middle panel) and lesion (right panel) groups.

Rats performed the task with high discrimination accuracy, particularly at the shorter stimulus onset times, where accuracy was consistently over 90%. However, at the two longest foreperiods, when left targets were less likely than right targets, the rats were more likely to make an incorrect choice and respond right following a left stimulus (Side x Foreperiod: $F(4,40) = 21.2$, $p < 0.001$, $\eta_p^2 = 0.68$, Observed power = 1.0). This was evident in the pre-surgery data and was also apparent in both control and lesioned animals post-surgery. This indicates that the changing spatial probability was perceived by the animal and they were anticipating the more probable target location.

6.4.4 Reaction time

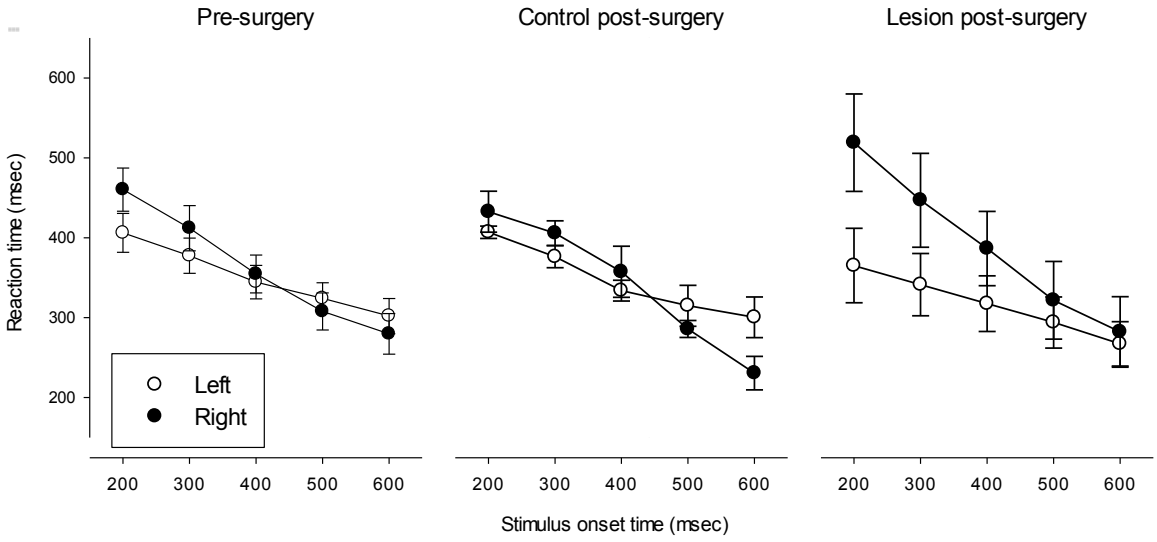


Figure 6.6. Line graphs depicting reaction time to right and left stimuli : left panel pre-surgery performance, middle panel post surgery sham, right panel post surgery lesion

Figure 6.6 shows the reaction time for correct responses pre-surgery (left panel), and the control sham-surgery (middle panel) and lesion (right panel) groups. Pre-surgery, reaction time was faster as a function of foreperiod, but also reflected stimulus spatial probability, such that at the shorter foreperiods reaction time was faster for the left responses and, at the longer foreperiods, reaction time was faster for right responses. This pattern remained for the control group after surgery. The lesion group however, no longer showed the same 'cross-over' effect of stimulus probability. Instead, the reaction time difference at the early foreperiods was exaggerated, with left response time speeded and right response time slowed. Although the difference reduced over time, even at the longest foreperiods, reaction time was not faster for right responses.

This was confirmed by a significant interaction of Group x Surgery x Side x Foreperiod ($F_{(4,40)} = 2.9$, $p < 0.05$, $\eta_p^2 = 0.22$, Observed power = 0.73) which was

subsequently analysed by restricted ANOVA to look for simple main effects of Group in the post-surgery data at each foreperiod on each side (Winer, 1986). The corrected F-ratios are shown in Table 6.2. For left responses, lesioned animals were faster than controls at the first two and final foreperiod. For right responses, they were slower than controls at all foreperiods.

Condition	Main effect of Group $F_{(4,40)}$ =	$p < 0.05$, $F_{(4,40)} > 4.08$ (*) $p < 0.01$, $F_{(4,40)} > 7.31$ (**) $p < 0.001$, $F_{(4,40)} > 12.61$ (***)
Left, 200ms	14.18	***
Left, 300ms	10.03	**
Left, 400ms	2.18	n.s.
Left, 500ms	3.58	n.s
Left, 600ms	9.06	**
Right, 200ms	60.71	***
Right, 300ms	13.84	***
Right, 400ms	6.96	*
Right, 500ms	10.54	**
Right, 600ms	21.7	***

Table 6.2. Corrected f ratios

Figure 6.7 shows the same post-surgery data as plotted in Figure 6.1, but plotted for each group by side of responses, to more easily compare the reaction times of each group. It is clear that the foreperiod-dependent speeding in reaction

time is equivalent for both groups on both sides because the lines are parallel. This indicates that they are sensitive to conditional temporal probability. It also indicates that they are sensitive to the changing spatial probability, because the slope of the lines are different for each side but not different as a function of group. The data indicate, however, that the initial left-side advantage / right-side disadvantage is exaggerated in the lesion group and this relative difference is not overcome throughout the foreperiod.

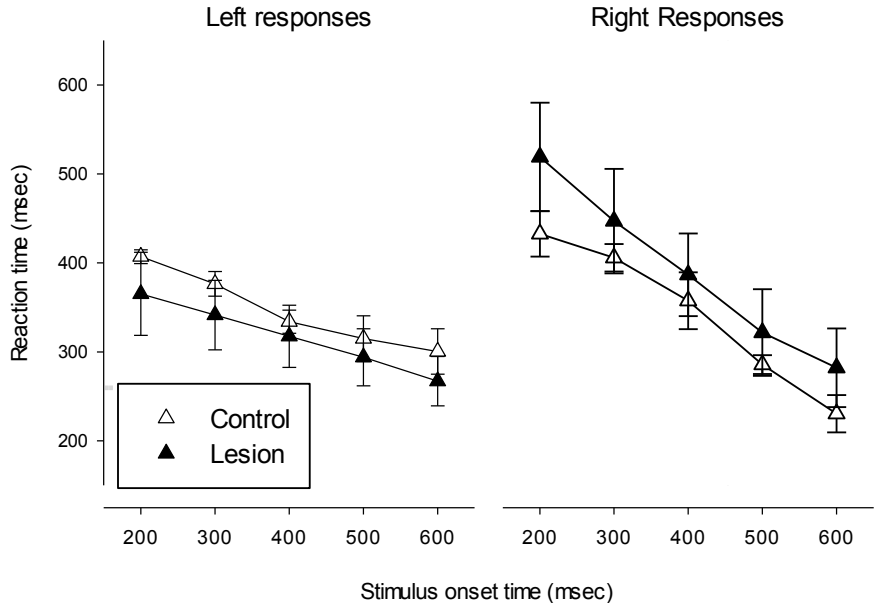


Figure 6.7. Line graphs depicting reaction time to right and left stimuli post surgery : left panel left responses across groups, right panel right responses across groups.

6.4.5 Reaction time Difference

Reaction time difference, which is reaction time to the most probable location minus reaction time to the least probable location, was additionally calculated.

6.4.5.1 Unilateral

There was little difference across any of the groups following unilateral surgery (see figure 6.8). The reaction time difference was greater at the earlier foreperiods (significant main effect of surgery [$F(1,9)=6.848$, $p=.028$]), although this was found to be consistent across all groups irrespective of lesion type and side (non- significant surgery x delay x group interactions [$F(8,36)=0.120$, n.s.).. Reaction time was quicker to more probable locations (left at earlier foreperiods and right at later foreperiods). This reaction time advantage was consistent with pre-surgery performance, thus suggesting that unilateral lesions did not impair the re-orientation of attention.

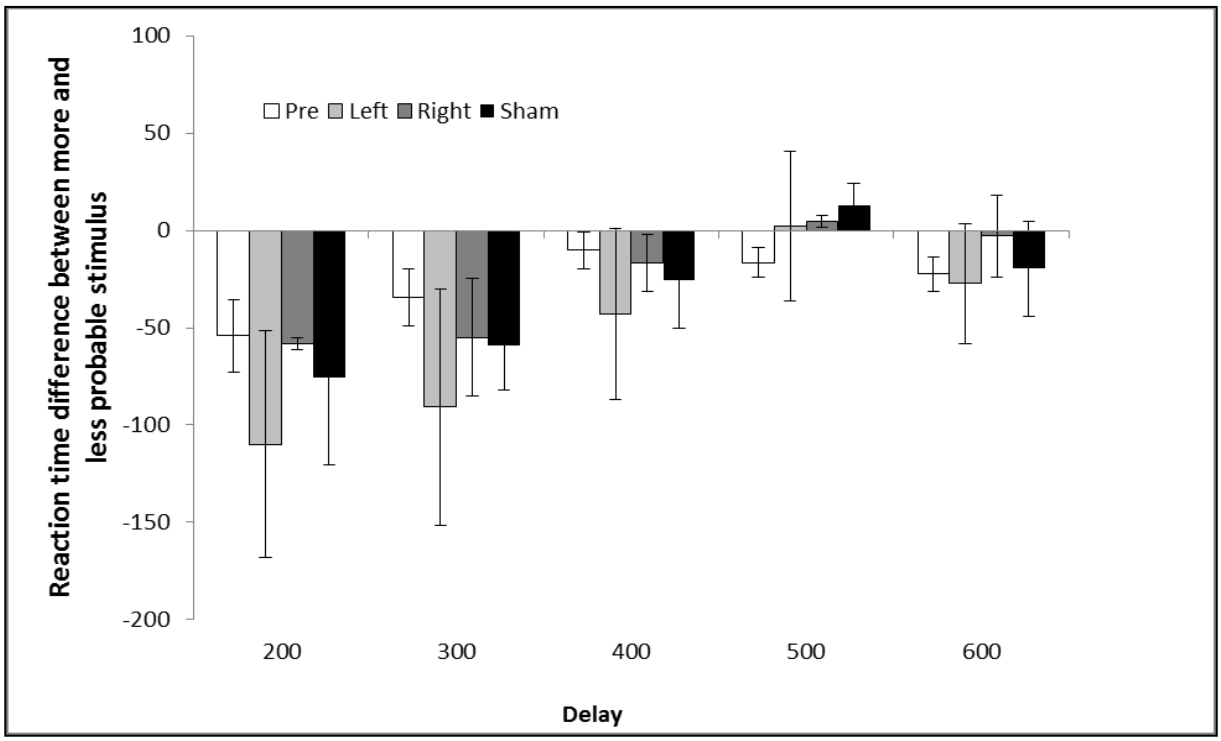


Figure 6.8. Reaction time difference (most probable minus least probable reaction time) after unilateral TRN 6OHDA surgery.

6.4.5.2 Bilateral

After bilateral surgery there was a big difference in reaction time difference profiles between the lesion group and the sham group (significant surgery x delay x group interaction [$F(4,40)=5.311$, $p<.01$]. See figure 6.9).

Sham surgery performance differed little from pre-surgery performance, with reaction time still being quicker to more probable locations. This suggests that the rats were able to re-orient their attention throughout the variable foreperiod to the site of target onset that they believed was more probable.

For the lesion group however, the reaction time advantage conferred to the left at earlier timepoints was maintained across all of the five foreperiods, even when right targets became more probable. The data suggests that the lesion rats took longer to re-orient their attention from right to left as the foreperiod increased. The rats appeared to focus attention to the left (more probable) side and did not re-orient their attention to the right as a function of the increasing probability of its appearance on the right.

The larger difference in reaction time at earlier foreperiods could be seen as a slowing in the re-orientation of attention from left to right side targets. Given the existence of the foreperiod effect, the reduction in this difference as the foreperiod increased can be explained by the general speeding of responses.

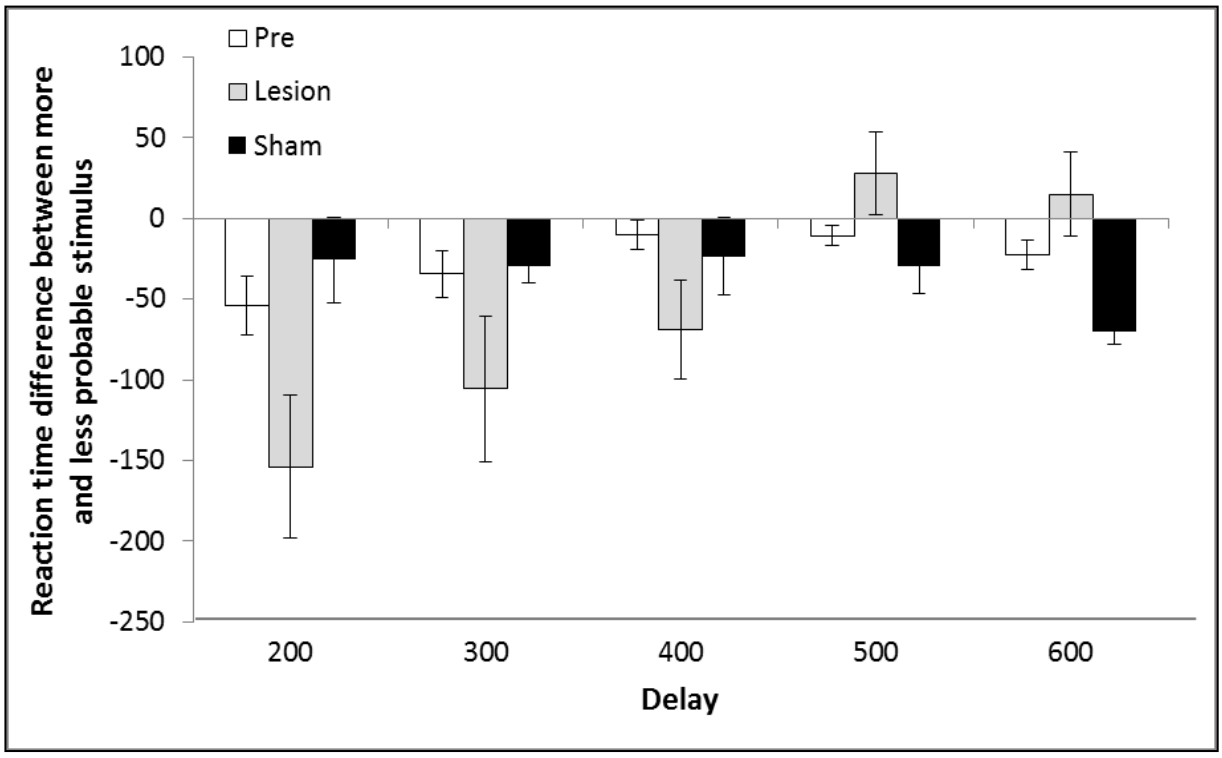


Figure 6.9. Reaction time difference (most probable minus least probable reaction time) before and after bilateral TRN 6OHDA surgery.

6.5 Discussion

The aim of the current experiment was to examine the effects of unilateral and bilateral 6-hydroxydopmaine thalamic reticular nucleus lesions in a test of top-down control of attention. It was predicted that depletion of dopamine in the TRN would result in alterations in the ability to disengage attention from a visuospatial location where attention had been previously been oriented. There was no effect of unilateral lesions on performance – reaction time advantage continued to reflect the increased probability of the side of target onset (quicker reaction time for left targets at earlier foreperiods and quicker reaction time for right targets at later foreperiods). However, consistent with our prediction we found that bilateral dopamine depletion in the TRN resulted in a consistent reaction time advantage for left targets throughout the variable foreperiod, even after right side targets became more probable. There was no impairment in reaction time as a consequence of the surgery, but rather a change in the speed of re-orientation of spatial attention and therefore the abolition of the advantage conferred by an increased probability for right targets during later foreperiods.

The lack of interactions between surgery and movement time demonstrate that any changes in behaviour should not be considered a function of altered motoric integrity. It was pertinent to establish this was the case in light of results presented by Anaya-Martinez, Martinez-Marcos, Martinez-Fong, Aceves, & Erlij (2006).The researchers examined the effects of unilateral 6-hydroxydopmaine thalamic reticular nucleus lesions on dopamine content and tyrosine hydroxylase positive (TH+) axons and terminals in the substantia nigra pars compacta, globus

pallidus and striatum of rats. It was shown that dopamine content and TH+ cells in the substantia nigra pars compacta was reduced by 68.2±1.7% and 61%±3.1%, respectively. Dopamine content in the striatum was reduced by approximately 40%, and by 60% in the globus pallidus. Depletion of striatal dopamine has been shown to induce significant motor impairments (Plowman, Thomas, & Kleim, 2011). Although striatal dopamine depletion results in overall slowing of reaction time in a task of covert orienting, there is no change in the ability to orient attention. This suggests that reaction time changes arose from impairment in response initiation rather than a motor impairment or an impaired directed attention (Ward & Brown, 1996). The protocol used by Anaya-Martinez and colleagues used 0.4µl of toxin, compared to our use of just 0.1µl. This could explain why they observed abnormal motor behaviour, namely circling, when the animals received TRN lesions. It is nonetheless important to consider the downstream knock on effects of lesions, and where possible take steps to determine whether these effects make significant contributions to findings.

A failure of unilateral lesions to produce a behavioural effect has been reported previously (Hefner, & Heffner, 1986; Blair, Cho, & Sharp, 1999; Schapiro, McClelland, Welbourne, Rogers, & Lambon Ralph, 2013). Take for example the case of patient HM. Left with significant memory impairments following bilateral hippocampal damage, Scoville and Milner (1957) stated aside from its theoretical significance, they published their results “a warning to others of the risk to memory involved in bilateral surgical lesions of the hippocampal region” (page 11). Discussing other cases they had shown that unilateral surgeries had few, if any, long term

implications for cognitive function. In light of this, finding a significant bilateral effect but not a unilateral effect is not surprising.

The present study demonstrated that rats with bilateral dopamine-depleting lesions of the thalamic reticular nucleus were able to discriminate and respond rapidly and accurately to visual stimuli. There is thus no evidence of a visual discrimination impairment.

In this task, the probability of target onset was manipulated such that it was more likely to appear on the left early in the trial and increasingly likely to appear on the right as the trial progressed.

The behaviour observed in this task in normal / control animals is that the likelihood of an error as well as response time (both reaction time and movement time) reflect the changing stimulus probabilities with time. An error is more likely, particularly at the longer foreperiods, when the animal is anticipating the more likely stimulus and the less probable stimulus is presented. Reaction times and movement times are also faster when a more likely stimulus is presented.

The faster reaction times result from both motor readiness as the rat prepares and anticipates the most likely response as well as covert attention to the likely stimulus onset location. For this reason, reaction times at the earlier foreperiods (when there has been less time to benefit from motor preparation) are slower overall that those at the longer foreperiods. With the stimulus probabilities changing with time, there is a point mid-way through the trial when each stimulus (hence each response) is equally likely. At this point in the trial, there should not be any performance differences in reaction time or percentage correct, which appears to be the case in the pre-surgery and control data, where the 'cross over point' is

close to 400ms. This remains true for the lesion group for all measures except for reaction time, which does not have a 'cross over point'. This indicates that the lesion does not entirely impair the ability to perceive the changing stimulus probabilities and adjust response preparation and attentional allocation in accordance. Nevertheless, there is an early left-side advantage when attention is allocated initially, at the start of the trial, which, notwithstanding the equally-efficient (compared to pre-surgery or to controls) re-allocation of attention, the initial differential is not overcome.

The results derived from this study have clear implications for disorders with a cognitive component and manifest aberrant dopaminergic function; namely schizophrenia and Parkinson's disease. Beyond dopaminergic manipulations, interest in the thalamic reticular nucleus as a locus of involvement in the schizophrenic phenotype is strong. Ferrarelli & Tononi (2011) published a recent review of evidence pertaining to the role of the TRN in schizophrenia. They presented various lines of evidence from genetics, psychopharmacology and sensory gating. As previously mentioned the atypical antipsychotic clozapine has a high affinity for D4 receptors and is unique in its ability to treat the cognitive symptoms of schizophrenia. Cognitive symptoms include disorders of attention, and given the role of the thalamic reticular nucleus in attention, many hypothesize a role of the TRN in the cognitive symptoms of schizophrenia.

Numerous studies have highlighted the presence of cognitive deficits in patients with Parkinson's disease (Owen, Sahakian, Hodges, Summers, Polkey, & Robbins,1995; 1995; Helie et al, 2012). Zhou, Chen, Wang, Yin, Hu, & Wang (2012) showed that early to moderate Parkinson's patients had difficulties re-orienting their

attention as compared to controls, with no difference in scores of alertness. Human studies are often marred by issues relating to medication use by patients as well as their stage of disease, but more controlled animal models for Parkinson's disease have confirmed the presence of cognitive deficits in species appropriate cognitive tasks (Bodis-Wollner, 2003).

We did not counterbalance the sides so that for all rats, the most probable location was left at earlier foreperiods targets with right becoming more probably with time. This was done to reduce the possibility of experimenter error. The side of the first surgery was counterbalanced however, but as there was no effect of the unilateral lesions, it seems unlikely that training to attend right at the early foreperiods would make any difference. It would be interesting to reverse the probabilities once stable post-surgery performance was present, to determine whether the animals could learn the new probabilities. Assuming the rats could learn that the contingencies had been reversed, it would be expected that they would show a bias to side where target onset was most probable at earlier foreperiods.

However, one also needs to consider using the same surgical procedure in other measures of attention. Although we can broadly say that we are using a task of attention, different tasks are able to measure different aspects of what we deem attention. It is therefore prudent that we attempt to ascertain whether the same lesion affects performance in other paradigms, such as the Posner valid/invalid cue task (Posner, 1980). It is possible that the lesion will result in an increased cost for invalidly cued targets as the rats would have difficulty in shifting their attention away from a location to which they had been previously directed to.

In summary, the results from the current experiment suggest not only that the thalamic reticular nucleus is involved in the orientation of attention – more specifically the speed at which attention is disengaged and moved towards a stimulus – but that dopamine activity is involved in this process. Widespread dopaminergic innervations seen throughout the brain in various segregated neuronal systems suggests that dopamine plays a significant role in the integration and modulation of cognitive, motor and limbic behaviour. Selective lesioning of the dopaminergic input to the thalamic reticular nucleus allowed us to examine the specific influence of dopamine on thalamic reticular nucleus function. Although further work is required to ascertain whether similar effects will be seen in different tasks of attention, it has nonetheless been shown that dopamine plays a key role in modulating both thalamic reticular nucleus function and the re-orientation of attention.

General Discussion

7.1. Overview

Chapter	Task	Brain region	Lesion manipulation	Effect
3	Cross modal distraction task	Thalamic reticular nucleus	Ibotenic acid lesion of visual thalamic reticular nucleus lesion	No effect of lesion on percentage correct when distractors were present
4	Attentional set shifting task	Thalamic reticular nucleus	Ibotenic acid lesion of thalamic reticular nucleus	No effect on acquisition of task or ID-ED shift
5	Attentional set shifting task	Mediodorsal thalamus and rostral thalamic reticular nucleus	Ibotenic acid lesion of mediodorsal thalamus or rostral thalamic reticular nucleus	No effect on acquisition of task or ID-ED shift
6	Spatiotemporal target probability signal reaction time task	Thalamic reticular nucleus	6-hydroxydopamine lesion of thalamic reticular nucleus	Increased reaction time advantage to left targets, even when less probable

Table 7.1. Summary of the manipulation and results from the experimental chapters detailed in this thesis.

This thesis has attempted to further reveal the role of the thalamic reticular nucleus in attentional processes. The location and shape of this nucleus in part explains the lack of behavioural research that has been conducted into its function. However, as discussed extensively in the introduction, those studies which have included a behavioural component strongly indicate that the thalamic reticular nucleus is involved in attention. Rather than responding merely to incoming sensory

information it is believed that the thalamic reticular nucleus acts as a filter. More specifically it is believed to serve as a filter not only at a sensory level but also at more cognitive levels. Organised topographic maps that mirror and project to sensory thalamic areas (visual: Coleman & Mitrafonis, 1996; auditory: Montero, 1983; somatosensory: Crabtree, 1992; gustatory: Hayama, Hashimoto and Ogawa, 1994) are hypothesised to be the mechanism through which the thalamic reticular nucleus 'gates' the flow of information from thalamus to cortex. The rostral sector is believed to be more involved with overarching cognitive processes such as learning and memory, due to its connectivity with regions such as anterior thalamus (Gonzalo-Ruiz, & Lieberman, 1995) and mediodorsal thalamus (Kuramoto, Pan, Furuta, Tanaka, Iwai, Yamanaka, Ohno, Kaneko, Goto, & Hioki, 2016) although the exact role played by these connections is yet to be fully established.

Crick postulated that the thalamic reticular nucleus acted like a spotlight – shining a light on certain aspects of the environment and therefore promoting their processing at the expense of other stimuli. Alongside maintaining attention the nucleus is believed to be involved in disengaging and shifting attention. Our experiment examining the role of 6-hydroxydopamine lesions supports this view. It was shown that dopamine depleting lesions which affected dopamine input to the thalamic reticular nucleus from the substantia nigra pars compacta. Following the lesions rats showed a failure to shift their attention towards the location which was statistically more likely to present the target. Their loss of the reaction time advantage to more probable locations suggests a failure to re-orient attention during the variable foreperiod – rather waiting for target onset to shift their attention and subsequently respond.

Given the proposed distinction in function across the rostral and caudal sectors of the thalamic reticular nucleus it was also hypothesised that lesions to the thalamic reticular would impair performance in the attentional set shifting task. The rostral sector is considered the more cognitive sector, and therefore an ideal candidate during this task. It had been predicted that lesioning this sector would result in an attentional set shifting deficit, with lesioned animals requiring significantly more trials than sham animals to reach criterion at extradimensional shift stage. However, as detailed in chapters 4 and 5 there was no effect of lesions of rostral and caudal sectors of the thalamic reticular nucleus on the acquisition of attentional set shifting rules, nor on the Id-Ed difference.

7.1.1 Chapter 3 – Thalamic blocking of distractors

Much of the hypotheses proposed pertaining to behavioural studies incorporating lesions to the thalamic reticular nucleus are based upon previous work using c-Fos markers (Montero, 1997; McAlonan, Brown, & Bowman, 2000; Petrof, & Brown, 2010), or non-behavioural electrophysological recording (Huguenard, & Prince, 1992; Troyano-Rodriguez, Lladó-Pelfort, Santana, Teruel-Martí, Celada, & Artigas, 2014). Although enlightening, these types of studies cannot shed light on the actual mechanisms through which the thalamic reticular nucleus is involved in attention. Further insight would be obtained should future studies replicate these experimental protocols with the addition of lesions.

For example, it had been hypothesised, based upon the McAlonan, Brown, and Bowman (2000) study that lesions of the visual thalamic reticular nucleus would impair the ability of the animal to block out the distracting auditory stimulus to

ensure optimal performance. The rationale behind this hypothesis was lateral inhibition. Studies have shown extensive inhibitory interconnectivity between thalamic reticular nucleus neurons (Shu, and McCormick, 2002; Landisman, Long, Beierlein, Deans, Paul, & Connors, 2002). One sector actively inhibiting another ensures enhanced processing of salient stimuli by limiting the allocation of cognitive resources to irrelevant stimuli. Increased Fos expression in the thalamic reticular nucleus sensory sector associated with the attended stimulus was hypothesised to represent this mechanism. However, results from our study using lesions and a distractor task did not reflect this mechanism – or rather the interruption of said mechanism. Had this mechanism been in effect it would have been expected that lesions in the sensory sector that dealt with the discrimination stimulus would result in significantly more errors being made when an auditory distractor was present. This would be due to the inability of the visual sector to inhibit the activity of the auditory sector. Being unable to limit attention paid to the distracting auditory stimulus should have resulted in poorer performance. However, these results were not found – there was no effect of the lesion on error rate performance.

There are several explanations for this – the first of which is that the thalamic reticular nucleus is not involved in attention and blocking out distractors. Electrophysiological studies have clearly that lateral inhibition within the thalamic reticular nucleus does exist (Lee, Patrick, Richardson, and Connors, 2014) showed some axons inhibit locally grouped neurons of the TRN, but others branch across the entire plane of the TRN to inhibit activity). Given what we already know about the physiological mechanisms of the thalamic reticular nucleus and cross sector inhibition, it seems unlikely that it is not involved in blocking out distractors.

Within this experiment rats were exposed to the distracting stimulus prior to receiving lesions. Performance before the distractors were introduced was very high, and while it dropped significantly they were still operating at around 90% correct. Had the rats not been exposed to the distractor before lesions were made, we may have seen different results. Going forward, it would be prudent to replicate the experiment but alter the point at which the animals are exposed to the distractors.

As discussed in the chapter's discussion, it is possible that the task did not assess top-down selective attention as such, and that bottom up control was sufficient to deal with the distractors. The relatively simple nature of the task, paired with the lack of salience attached to the distractors may have made top-down input unnecessary. It would be interesting to see whether increasing the cognitive load associated with the task would recruit the thalamic reticular nucleus and therefore make the lesion detrimental to performance. Importantly, the results suggest a distinction between filtering of relevant sensory information and blocking out distractors – something that warrants further study.

As already mentioned, it would be very interesting to see the effects of lesions on the blocking paradigm. Although McAlonan, Brown, and Bowman (2000) proposed the mechanism of lateral inhibition, and we based our hypotheses on the same mechanism, the two experiments are very different. The blocking experiment relies heavily upon conditioning, requiring little discrimination or cognitive load from the rats. The rats are not expected to make different responses depending upon stimulus presentation –having to respond in the same manner when the stimulus appeared. The distractor task, as a two alternative forced choice task required two responses while actively ignoring the distractor stimulus. Therefore, while it is

expected they both require active inhibition of a sensory sector, the exact mechanisms are not the identical. Therefore, producing lesions alongside the blocking paradigm would help clarify the situation regarding the mechanisms potentially identified within the McAlonan, Brown, and Bowman (2000) study.

7.1.1.1 Chapters 4 and 5– lack of thalamic involvement in attentional set-shifting

Lesioning both rostral and caudal sectors of the thalamic reticular nucleus, in addition to the mediodorsal thalamus, were found to not impair performance on the attentional set shifting paradigm.

Discussions of the role of the thalamic reticular nucleus in attentional set shifting in chapter 4 are complicated by the lack of attentional set formation. Although lesion animals did not differ from control animals, they did not show the pattern of behaviour expected. Given that control animals also failed to form attentional set, it is difficult to conclude whether the lesion had any effect. The study woiuld need to be repeated, perhaps using multiple tests until attentional set was demonstrated, which would allow for more definitive conclusions to be drawn about thalamic reticular nucleus involvement.

The connectivity between the rostral thalamic reticular nucleus and other cognitive areas such as anterior thalamus (Gonzalo-Ruiz, & Lieberman, 1995) and mediodorsal thalamus (Kuramoto, Pan, Furuta, Tanaka, Iwai, Yamanaka, Ohno, Kaneko, Goto, & Hioki, 2016), and hippocampus **(**Çavdar, Onat, Çakmak, Yananli, Gülçebi, & Aker, 2008) make it an ideal candidate for studying cognitive involvement.

Unlike the sensory sectors, the rostral sector is implicated in higher level functions. However, there are few studies that have examined the rostral thalamic reticular nucleus in an extensive manner.

Vann, Brown, and Aggleton (2000) found increased fos expression in the rostral sector of the thalamic reticular nucleus in the more demanding iterations of their spatial working memory task using an 8 arm radial maze. This study does not provide us with any information as to the functional contribution of the thalamic reticular nucleus to spatial working memory, so we cannot fully draw conclusions from the paper. One could suggest that increased activity of the thalamic reticular nucleus in some of the tasks detailed reflects a more general modulation of consciousness and cognitive arousal rather reflecting a more specific role in spatial memory. This would be consistent with Wilton, Baird, and Muir (2001) who failed to show an effect of rostral thalamic reticular nucleus lesions on spatial memory performance. While the other sectors of the thalamic reticular nucleus have specific sensory projections, for example visual sector to lateral geniculate nucleus, the rostral sector has projections to regions with less specificity. It is possible that these regions, in conjunction with other cortical and subcortical areas are involved in global arousal. This would fit in with thalamic reticular nucleus involvement in epilepsy, and the loss of consciousness/alterations in global arousal (Van de Bovenkamp-Janssen, Akhmadeev, Kalimullina, Nagaeva, Van Luijtelaar, & Roubos, 2004). Perhaps, rather than having a direct role in specific attention\cognitive functions, the rostral sector of the thalamic reticular nucleus is involved in synchronising thalamocortical oscillations. This also ties in with the role of the

thalamic reticular nucleus in the generation of sleep spindles and transition between an awake and non-awake state.

It has been established that stimuli with strong positive or negative affective connotations can bias attention and the allocation of other cognitive resources. Studies have recently shown that there are connections between the thalamic reticular nucleus and the amygdala in primates (Zikopoulos and Barbas, 2012). It was shown that projections from the amygdala terminated in those regions with strong connectivity with the mediodorsal thalamus and orbitofrontal cortex. This converging emotional and attentional circuit is interesting, and could perhaps capture the lack of performance deficit following lesions. Without a strong emotional component to the task, it is possible that the non-sensory sector of the thalamic reticular nucleus was not recruited for performance. The authors suggest that this circuit is used most during high cognitive demand and where salient stimuli have a strong affective component. It is well established that emotional stimuli grab attention (Pool, Brosch, Delplanque, & Sander, 2016) and may have been required for the rostral thalamic reticular nucleus to be involved in this task.

The lack of olfactory representation in the sensory sectors of the thalamic reticular nucleus is problematic, and does not permit one to study the allocation of attention towards sensory aspects of the task. Our data suggests that the thalamic reticular nucleus is not involved in the shifting of attention, nor reversals on a more global level. However, we do know that the thalamic reticular nucleus is involved in the re-orienting of attention towards stimuli which are predictive of reward (chapter 6). Animals had been trained to associate the light presentation and their subsequent response with a food reward, thus making the light stimulus highly

salient. Lesioning the thalamic reticular nucleus meant that animals failed to use top down control to guide attention towards the location where the stimulus was most likely to appear.

7.1.1.2 Chapter 6 – reducing dopamine in the thalamic reticular nucleus impairs the re-orienting of attention

Changes in the shifting/re-orienting of attention in a top down attentional task following dopamine depleting lesions in the thalamic reticular nucleus fit with early proposal made by Crick (1984). One of the criteria for the attentional spotlight hypothesis was that it should be able to disengage and switch attention towards more salient stimuli, or in our case more predictive location. By reducing dopamine input into the thalamic reticular nucleus the rats took significantly longer to shift their attention from a previously predictive location towards a currently predictive location. The rats had extensive training in the task and so were familiar with the protocol. Furthermore, it could not be argued that they could no longer appreciate the variable foreperiod because there was still a main effect of foreperiod. Rather, it seems that they failed to appropriately re-orient their attention – relying on stimulus onset to shift. These results are consistent with the data from Weese, Phillips, and Brown (1999) who found that lesions of the visual thalamic reticular nucleus abolished the validity effect in the Posner task. Rather than using the cue light to orient their attention, the rats only oriented their attention when the target light appeared. This is evident given the lack of reaction time detriments when an invalid cue was presented. These data suggest that the thalamic reticular nucleus is intrinsically involved in orienting and re-orienting attention towards biologically

relevant stimuli which are linked with the presentation of a food reward (assuming the proper action is taken).

Disruptions to the orienting attention network has also been shown in patients with Parkinson's disease. Zhou, Chen, Wang, Yin, Hu, and Wang (2012) studied both Parkinson's patients and healthy controls using the attention network task. The task measures alerting, orienting and executive control of attention. It was found that Parkinson's patients had severe impairments in the orienting and re-orienting of attention in a forced choice reaction time task. Consistent with the data from chapter 6 there was no overall impairment in alerting and executive control of attention. The patients in the study were in the earlier stages of the disease, which may account for the lack of global attention deficits. Dopamine degeneration seen in chapter 6 was not as severe as that seen in the late stages of the disease, and could explain the similar results. Reduced dopamine concentrations in the substantia nigra pars compacta of Parkinson's patients has been well documented (Forno, 1996; McNaught, Belizaire, Jenner, Warren-Olanow, Isacson, 2002; Gröger, Kolb, Schäfer, & Klose, 2014) with even early stages of the disease showing a profound loss of tyrosine hydroxylase positive neurons. We did not find movement time impairments, indicative of intact motoric system. Given that motor deficits are a hallmark of Parkinson's disease this cannot be considered a model of the disease (Dujardin et al, 2013). However, these results are of interest when studying the cognitive deficits in the disease and implicate thalamocortical dysfunction in the progression of attention deficits.

Another application of studying the cognitive capacity of the thalamic reticular is with regards to schizophrenia. Although schizophrenia is a complex disorder with

several proposed etiologies ranging from aberrant development to genetic predispositions, many suggest a role for thalamic structures in the cognitive symptoms. Disrupted prefrontal-thalamic connectivity has been shown to be present in schizophrenic patients (Woodward, Karbasforoushan, & Heckers, 2012) with these aberrant connections suggested to account for many of the cognitive symptoms of the disease. The thalamic reticular nucleus serves to gate the flow of information from thalamus to cortex. Disruption of this mechanism could easily explain abnormal transmission of sensory information from thalamus to cortex, and therefore the imaging data available. Studies have focused mostly on sensory thalamus, rather than the thalamic reticular nucleus. Given that the involvement of the thalamic reticular nucleus has once again been shown to be involved in attention, it is an ideal candidate to study with regards to schizophrenia. During development the disrupted in schizophrenia 1 (DISC1) gene is expressed in the thalamic reticular nucleus fitting with theories of abnormal thalamic development and later development of schizophrenia.

Furthermore, studies into sleep disturbances suggest a pivotal role for the thalamic reticular nucleus (Young and Wimmer, 2016; Ferrarelli, and Tononi, 2016). However, the behavioural evidence pertaining to a cognitive role is less substantial. It would be particularly interesting to examine the thalamic reticular nucleus in various rodent models of schizophrenia. We have shown a cognitive role for the thalamic reticular nucleus when reducing dopamine input. By increasing dopaminergic activity in the thalamic reticular nucleus the data would provide a detailed picture of the contribution of the neurotransmitter to thalamic dependent attention. Examination of rodent models could range from simple neuroanatomical

dissection to see any morphological differences, as well as electrophysiological recording both in and ex vivo. Many of the developmental models of schizophrenia have neuroanatomical changes that mirror those deviations seen in schizophrenia, so examining the thalamic reticular nucleus in these models could provide a great deal of evidence.

7.2 Different types of attention

William James poignantly stated “everyone knows what attention is” (James, 1890). Although it is a statement that has been used hundreds, if not thousands, of time within the literature, it is a very simple statement. While we could say that the layperson knows what attention is in the general terms, its use in scientific examination has many different interpretations/iterations. The use of the term attention varies across contexts and disciplines. Therefore, to say the thalamic reticular is involved in attention would be a misleading statement. This thesis has shown that the thalamic reticular nucleus is involved in certain aspects of attentional control, but it would be naïve to believe it is involved in all aspects of attention. For one, although the thalamic reticular nucleus does have widespread connections with cortical and subcortical structures (Pinault, 2004), it is not directly connected with all areas also involved in attention. It is therefore pertinent to discuss the different types of attention within the context of our research and the thalamic reticular nucleus.

7.2.1 Bottom up and top down attention

It is perhaps best to first differentiate between bottom-up and top down attentional processes. Bottom up processing is viewed as a more crude level of processing, driven by the presence of stimuli within the environment (Hunt & Kingstone, 2003). For example, the re-direction of attention from a task to a fire alarm currently set off. Top down attention on the other hand, is a more refined processing of information driven by more complex cognitive processes, relating to previous knowledge or goals and Is therefore slower to engage (Baluch, & Itti, 2001)

We must be careful not to reinforce the dichotomy between bottom-up and top-down too much, as tasks and everyday life require a balance between the two. Although cognitively demanding tasks often require top down control of attention, sensitivity to non-task related stimuli is also necessary – a failure to shift attention towards a salient stimulus could be just as problematic as an inability to maintain attention towards a task. The terms bottom-up and top-down should be considered conceptualisations rather than anatomical realities – areas involved in bottom up or top down attention are not necessarily mutually exclusive (Sarter & Bruno, 2001). At the point of data collection (rather than collection of training data to reach predetermined performance criteria) in our 6OHDA task top down attention was being studied. More specifically, we were studying focused/ sustained attention. The rats were aware of the contingencies of the task, knew the spatial location that attention needed directed towards, and importantly knew the response needed to be made when the target appeared.

Sustained attention is often viewed as the means of ensuring adequate performance from distractors. By ensuring that attention and resources are focused

on relevant task contingencies, irrelevant stimuli can be marginalised. In chapter 6 we did not introduce any distractors into the task, instead choosing to study orientation and shifting of attention.

7.3 Future directions

One of the main barriers facing researchers when attempting to study the thalamic reticular nucleus at a behavioural level is the difficulty of producing focal lesions. As it is such a thin nucleus, just a tiny deviation can result in adjacent areas being lesioned rather than the target. When faced with ethics boards and the need to reduce the number of animals used in scientific experiments many understandably are choosing to forego studying this important area. Fortunately, new technological and scientific advances are making it easier for researchers to target specific areas with more accuracy. One of these new advances is optogenetics. As is customary, new techniques see a replication and revival of old studies and protocols. What is novel with the thalamic reticular nucleus is that new techniques offer us a way to rectify the paucity of behavioural studies. In addition to extending knowledge gained from the limited number of lesion studies available, we now have the opportunity to study the thalamic reticular nucleus in a much more reliable manner.

It is clear that this relatively new technique provides great opportunity for researchers studying the thalamic reticular nucleus.it would be particularly interesting to replicate Fos studies using an optogenetic approach to better understand the role played during various stages of the tasks. Optogenetics permits a temporal control of disruption without producing lasting effects, which presents

fantastic opportunities for researchers. A better understanding of the circuits the thalamic reticular nucleus is involved in will provide great insight and promote more informed investigation. To date, behavioural studies are involved only by a handful of other studies, many key studies decades old, or else non-behavioiural slice electrophysiological studies which by their very nature are artificial and offer no "real world" insights into how function relates to behaviour.

The c-Fos experiment by Montero (1997) showed increased activation of the thalamic reticular nucleus sensory sector required when exploring a novel environment (visual for typical rats and somatosensory for blind rats). As we now have the capabilities to measure activity in freely behaving animals we could measure activity in the relevant sectors of both the thalamic reticular nucleus and dorsal thalamus both at the start and during exploration. If visual cues provided the best information about the environment then one would assume that after an initial period of attention to all sensory cues, the visual sector would then suppress some of the activity of other areas. It is possible that olfaction would be the main sensnory cue used, but with no olfactory representation in the thalamic reticular nucleus this was not measured within the Montero study. While a relatively simple study, it would serve to provide more clarification as to the mechanisms behind the study. Indeed optogenetics could be also employed.

Our research to date has only studied one neurotransmitter system in any detail. Dopamine's pivotal role in both schizophrenia and Parkinson's disease, and the suggested role of the thalamic reticular nucleus in the cognitive deficits in the former (and to some extent the latter) were decisive in its inclusion within this thesis. Also of interest however is the cholinergic system. The data in this thesis has

shown subcortical involvement in attention, and more specifically subcortical dopaminergic input. Basal forebrain cholinergic transmission has been shown to be involved in attentional processes (Voytko, Olton, Richardson, Gorman, Tobin, Price, 1994; Everitt, Robbins, & Muir, 1995; Everitt, & Robbins, 1997; Waite, Wardlow, & Power, 1999; Sarter, Bruno, & Givens, 2003). Tracing studies have shown that the basal forebrain projects to thalamic reticular nucleus (Steriade, Parent, Pare, & Smith,1987; Jourdain, Semba, & Fibiger, 1989), with recent electrophysiology studies showing that postsynaptic activation of nicotinic acetylcholine receptors triggers action potentials in thalamic reticular nucleus neurons (Sun, Pita-Almenar, Wu, Renger, Uebele, Lu, & Beierlein, 2013). Systemic administration of the cholinergic agonist nicotine and antagonist scopoloamine were found to differentially affect reaction time to valid and invalid cues in a reaction time task (Phillips, McAlonan, Robb, and Brown, 2000). Although systemic administration does not provide causal evidence as to the exact cholinergic mechanism(s) in effect, mechanisms involving the thalamic reticular nucleus are a strong candidate. With cholinergic control of thalamic activity established, the thalamic reticular nucleus would explain the results found by Phillips, McAlonan, Robb, and Brown (2000) whereby agonists reduced reaction time and antagonists increased reaction time to invalid cues in the task. This is of course speculative, but given the knowledge we already have of the thalamic reticular nucleus, it warrants further examination.

7.4 Conclusions

This thesis has attempted to elucidate the functional role of the thalamic reticular nucleus In cognition, more specifically attention. This thesis details the use

of excitotoxic and dopamine depleting lesions to perturb thalamic reticular nucleus function. Employed alongside reliable behavioural paradigms the experiments documented have highlighted the role of the thalamic reticular nucleus in awake, freely behaving animals. Consistent with previous research, it has been shown that the thalamic reticular nucleus is not just a passive relay for sensory information but rather has a critical role in the top-down re-orienting of attention.

It was shown that dopaminergic input into the thalamic reticular nucleus from the substantia nigra thus pars compacta is intrinsically involved in re-orienting attention. Critically, this extends the proposals that the thalamic reticular nucleus is involved in the cognitive pathologies seen in psychiatric/neurodegenerative disorders such as schizophrenia and Parkinson's disease. Earlier studies have shown how thalamic reticular nucleus dysfunction can account for the sleep disturbances seen in these disorders, and this thesis provides additional evidence for its role in cognitive issues.

In conclusion, this thesis confirms that subcortical mechanisms of attentional control and selectivity critically include thalamic reticular nucleus function, and that dopamine is invariably involved.

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